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Method of producing double low restorer lines of brassica napus having a good
agronomic value

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The invention relates to a method of producing a double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting a radish introgression carrying the Rfo restorer genes deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good
5 agronomic value characterized by female fertility, a good transmission rate of Rfo and a high vegetative vigour. The invention relates also to a method of forming Brassica napus hybrid seed and progeny thereof and to the use of markers for selection.

Breeding restorer lines for the Ogu-INRA Cytoplasmic Male Sterility (cms) system
10 in rapeseed (Brassica napus L.) has been a major objective during the past few years. Extensive backcross and pedigree breeding were necessary to improve their female fertility and to get double low restorer lines. The so-called « double low » varieties are those low in erucic acid in the oil and low in glucosinolates in the solid meal remaining after oil extraction. However some difficulties can still be
15 encountered in breeding these lines (introgression rearrangements, possible linkage with negative traits) due to the large size of the radish introgression.

The inventors thus assigned themselves the objective of providing a new improved double low restorer line with a good agronomic value.

This objective is obtained by a new method of producing a recombined double low
20 restorer line for the Ogu-INRA cms in rapeseed.

A first object of the present invention relates to a method of producing double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having
25 a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, said method including the step of:

- a) crossing double low cms lines of spring Brassica napus comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of Brassica napus,
- 30 b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation,

- c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line,
 - d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,
 - 5 e) selecting progeny lines.
- A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.
- According to one advantageous form of embodiment of the method according to the present invention, the double low cms line of spring Brassica napus of step a) is
- 10 R211.
- R211 is an INRA spring restorer line.
- Drakkar is a French spring registered variety.
- Wesroona is an Australian spring registered variety.
- According to one advantageous form of embodiment of the method according to the
- 15 present invention, the testing is performed with the combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.
- Another object of the present invention relates to double low restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a
- 20 good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.
- According to one advantageous form of embodiment, the double low restorer lines present a unique combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.
- 25 Another object of the present invention relates to a method of forming Brassica napus hybrid plants and progeny thereof obtained through the steps of:
- a) providing a restorer line produced according to claim 1 and bred to be homozygous,
 - b) using said restorer line in a hybrid production field as the pollinator,
 - 30 c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant, and
 - d) harvesting the hybrid seed from the male sterile plant.

Another object of the present invention relates to seeds of Brassica plant obtained from the methods according to the present invention.

Still another object of the invention relates to seeds of Brassica napus deposited in NCIMB Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on
 5 July 4, 2003, under the reference number NCIMB41183.

Another object of the present invention relates to the use of at least four markers PGIol, PGIint, BolJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombined restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and
 10 recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

In a preferred embodiment, the combination is of five markers PGIol, PGIUNT, PGIint, BolJon and CP418.

15 In the present invention, the expression " any portion of them comprising at least one polymorphic site" means any part of the sequence showing at least a difference between the B.oleracea type sequence and B.rapa type sequence.

Such markers are represented in the following figures and sequence listing for the R2000 line.

20 According to one advantageous form of embodiment, the present invention relates to:

- The marker PGIol which is amplified using the primers: PGIol U and PGIol L
 (PGIol U: 5'TCATTGATTGTTGCGCCTG3';
 PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')
- 25 - The marker PGIint which is amplified using the primers: PGIint U and PGIint L
 (PGIint U: 5'CAGCACTAATCTTGCGGTATG3';
 PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
- The marker PGIUNT which is amplified using the primers: PGIol U and PGIint L:
 (PGIol U: 5'TCATTGATTGTTGCGCCTG3';
 30 PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
- The marker BolJon which is amplified using the primers: BolJon U and BolJon L:
 (BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';

BolJon L: 5'GCCTACTCCTCAAATCACTCT3')

- The marker CP418 which is amplified using the primers: SG129 U and pCP418 L:

(SG129 U: cf Giancola et al, 2003 *Theor Appl. Genet.* (in press))

pCP418 L : 5'AATTTCTCCATCACAAGGACC3')

5 Another object of the present invention relates to the PGIol, PGIUNT, PGIint, BolJon and CP418 markers whose sequences follow:

PGIol R2000 marker:

	TCATTTGATT	GTTGCGCCTG	TCGCCTTGTT	GTGTTATGAT	GAATGAACAG	CAGTCATTTA	60
	ACATGTGGTT	AACTTAACAG	GGCTCCGGCT	GTTGCAAAAC	ACATGGTTGC	TGTCAGCACT	120
10	AATCTTGCGG	TATGAATTTG	TGATTAAATT	TGTTTGTTTG	TGACTCTTTC	TTCATTGTTC	180
	GTTTTCGTAC	AATAAACCGA	ATGTATAATC	TTTTTACAAA	CTGAATTTTC	TACCGGGTCT	240
	GATGTACA						248

PGIUNT R2000 marker:

15	TCATTTGATT	GTTGCGCCTG	TCGCCTTGTT	GTGTTATGAT	GAATGAACAG	CAGTCATTTA	60
	ACATGTGGTT	AACTTAACAG	GGCTCCGGCT	GTTGCAAAAC	ACATGGTTGC	TGTCAGCACT	120
	AATCTTGCGG	TATGAATTTG	TGATTAAATT	TGTTTGTTTG	TGACTCTTTC	TTCATTGTTC	180
	GTTTTCGTAC	AATAAACCGA	ATGTATAATC	TTTTTACAAA	TGAATTTTCT	ACCGGGTCTG	240
	ATGTACAATG	CTAGTCTCCA	TGTTCTTGCG	GATCATGATT	TATTTTCTAC	ATGTATTTCAG	300
20	ACAGTACAGA	AGAAAGTGTT	CAAAACTCTG	GATGTTTTAA	TTTACAGTTA	GTGGAGAAGT	360
	TCGGCATTGA	TCCGAACAAT	GCATTTGCAT	TTTGGGACTG	GGTTGGTGGG	AGGTACAGTG	420
	GTAAGTGCTT	GTTTATTTGG	TTGTATAAAT	TTCTCGTCCA	TTTCCGCTTG	CTTAGTGTAT	480
	AACTGAAATT	CTTTTGCAAT	TTGCAGTGCT	GTTGGAGTCT	TACCATTGTC	TCTACAGTAT	540
	GGCTTCTCTG	TGGTTGAGAA	GTACGGTACC	TTCTACTTTA	TCAGCCATCT	CATAAAATGT	600
25	CTTAGGCATA	TTCTTTCTAT	TTTATTTCCC	TCTTAATGAT	TTCTTCTTTT	TTTTATTGCA	660
	TTCCCCTTTT	ATTTTCAAAA	GTTGTTACTG	TCTCTAAATC	AAGAAGAAAC	CTTCTTAGTA	720
	GATCCAGCTG	ATATTACAGC	TTTTTTAAAT	TGGACTGCAG	GTTTTTAAAG	GGGAGCTTCA	780
	AGCATTGATA	AGCATTTCCT	GTCCACACCG	TTTGAGAAGA	ATATACCCGT	GAGTTGCATT	840
	AGTTGTGTGA	TTATACAGTT	TTCTTGTCTT	TTTGCTATGT	CCATCAACAC	TAGAGATTCG	900
30	TGAAGTTATT	AGTGTAGTCA	ACGCATAGGG	AGAGGTGATT	GGTGACTTTT	GGACGATTTC	960
	AGGTGCTTTA	GGGTTATTG					979

PGIint R2000 marker:

35	CAGCACTAAT	CTTGCGGTAT	GAATTTGTGA	TTAAATTTGT	TTGTTTGTGA	CTCTTTCTTC	60
	ATTGTTTCGT	TTCGTACAAT	AAACCGAATG	TATAATCTTT	TACAAACTGA	ATTTTCTACC	120
	GGGTCTGATG	TACAATGCTA	GTCTCCATGT	TCTTGGGGAT	CATGATTTAT	TTTCTACATG	180
	TATTCAGACA	GTACAGAAGA	AAGTGTTCAA	AACTCTGGAT	GTTTTAATTT	ACAGTTAGTG	240
	GAGAAGTTTC	GCATTGATCC	GAACAATGCA	TTTGCAATTT	GGGACTGGGT	TGGTGGAAGG	300
	TACAGTGGTA	AGTGCTTGTT	TATTTGGTTG	TATAAAATTC	TCGTCCATTT	CCGCTTGCTT	360
40	AGTGATATAAC	TGAAATTTCT	TTGCAGTTTG	CAGTGCTGTT	GGAGTCTTAC	CATTGTCTCT	420
	ACAGTATGGC	TTCTCTGTGG	TTGAGAAGTA	CGGTACCTTC	TACTTTATCA	GCCATCTCAT	480
	AAAATGTCTT	AGGCATATTC	TTTCTATTTT	ATTTCCCTCT	TAATGATTTT	TTCTTTTTTT	540
	TATTGCATTTC	CCGTTTTATT	TTCAAAAGTT	GTTACTGTCT	CTAAATCAAG	AAGAAACCTT	600
	CTTAGTAGAT	CCAGCTGATA	TTCAGCCTTT	TTTAAATTTG	ACTGCAGGTT	TTTAAAGGGG	660
45	AGCTTCAAGC	ATTGATAAGC	ATTTCCAGTC	CACACCGTTT	GAGAAGAATA	TACCCGTGAG	720
	TTGCATTAGT	TGTGTGATTA	TACAGTTTTT	TTGTCTTTTT	GCTATGTCCA	TCAACACTAG	780
	AGATTTCGTGA	AGTTATTAGT	GTAAGTCAAC	CATAGGGAGA	GGTGATTGGT	GACTTTTGGA	840
	CGATTTCAGG	TGCTTTAGGG	TTATTG				866

BolJon R2000 marker:

	GATCCGATTC	TTCTCCTGT	GAGATCAGCT	CCAAACATCA	AACAACCTTGT	ACACAAATAT	60
	CTTTACTTGC	TAAATGGAAC	ATGACAAGAG	ATAGAAAATC	TTGCTCATAG	TATTGTACAA	120
	GGGATAACAG	TGTAGAAAAC	AAACCGTCTG	TAAGATTTTC	TCCCTGATCC	TCTCACTTAA	180
5	CCAGTAGGCG	TTTTTCACAT	TGAAGCGCAT	ATCTACTTTG	GTATTCACCTG	AATAAAAAAA	240
	GAAAGCTGGT	AACATGTGAA	GGATATACAA	GCATTGATAC	ACCAAGTAGT	CACAAACTAC	300
	ATTATAAAGG	TCAGACCTTT	GTTACACATC	TGGCCTCCAG	GACCACCGCT	TCTAGCAAAG	360
	TTAAGCGTAA	CATGGTCTGC	ACGTATACAA	ATGAAAATGT	TTCTATCAAA	ATCCTATAAA	420
	ATAGAGCTCT	ATAACATTGT	CGATACATAG	TTTCACTAAC	TCTGCAAGTA	CTAAACACAT	480
10	ATACAAACAA	AACTATGCGA	ACAGATCAAA	ACTACTACAG	AACACAGTTC	TATGACACTG	540
	TCGATAGTAA	CATCCTCTGC	AAGTACCAAA	GAGATAGCAA	ATGAAACTAT	GTAAACAAAT	600
	CAAAATTCTA	AATTTCTCCA	TCACAAGGAC	CTACAGAATA	GAGTTATCAT	AACATTTTCT	660
	GTAAATATTT	CCATCAAAAT	GACTAGAGAA	CAGAGTTCTT	ATAACATTAT	CTGTAAATGT	720
	TCCAACAAAA	CCACTACATA	GCAGAGTTCT	TATAACATTG	TCTGTAAATG	TCCAATCAAA	780
15	ACCACTACAG	AACAAAGCTC	CTATAACATT	GTTTATACAA	AGTTTCACTA	AATCTACAAA	840
	CTTTCCCCGT	AAATGAGCTT	AATATCACCC	AAAGATGTTT	CAATCAGATA	AAGAGTACGA	900
	CATCGTTTTG	AGATTAGAAC	AAACTGAAAC	TTACGTAGAG	TGATTTGAGG	AGTAGGC	957

CP418L R2000 marker:

20	AATTTCTCCA	TCACAAGGAC	CTACAGAATA	GAGTTATCAT	AACATTTTCT	GTAAATATTT	60
	CCATCAAAAT	GACTAGAGAA	CAGAGTTCTT	ATAACATTAT	CTGTAAATGT	TCCAACAAAA	120
	CCACTACATA	GCAGAGTTCT	TATAACATTG	TCTGTAAATG	TCCAATCAAA	ACCACTACAG	180
	AACAAAGCTC	CTATAACATT	GTTTATACAA	AGTTTCACTA	AATCTACAAA	CTTTCCCCGT	240
	AAATGAGCTT	AATATCACCC	AAAGATGTTT	CAATCAGATA	AAGAGTAACG	ACATCGTTTT	300
25	GAGATTAGAA	CAAACTGAAA	CTTACGTAGA	GTGATTTGAG	GAGTAGGCTC	GTTGCCAGCA	360
	GAGCTAGCTC	TCTCCTCCGC	CTCATGAAGC	ATCTGTTGCA	CCTGAGACAA	CCGTGACGAA	420
	ACTTTCCGAT	CACCGCCACC	AGAATTCGAC	GCCGCGCATC	GGAAGGATCC	GAATCGGGAA	480
	CTGAGTGAAC	CCGAGCGATC	CCGGGAGTGC	GACGGAGCGA	TGGGAAAAGA	GAGTGGCAGC	540
	ATTTTCGACGA	AGAGTGGAAG	AGGAGAGGGT	GGTGGATAAA	CTCGCGTATG	ATCAAGTTCCG	600
30	TCATCGTCCT	GATTGCCGCC	ATTTTTTTTG	TCAGGGCGCT	CTGTGGCTTA	GAAGTTTCCG	660
	ATGTCAATGA	AC					672

In the annexed drawing that follows, the following abbreviations are used :

	Dra	Drakkar
35	Rel-15-1, E38,R15	R2000
	Hete, Hel, R211.Drakkar	heterozygous R211*Drakkar,
	Darm	Darmor
	Bol:	Brassica oleracea
	Bra, B.rap:	Brassica rapa
40	GCPA18-A19, Wes, Aust:	Wesroona
	Sam, SamlPGIolSunt5	Samourai
	RRH1, ba2c	RRH1
	rav, N.WR	Hybrid Brassica napus*wild Radish

- Figure 1 illustrates Gamma ray Irradiation and F2 production.

45 - Figure 2 illustrates seed set on 'R211' and 'R2000'.

- Figure 3 illustrates the number of seeds per pod of different lines.

- Figure 4 illustrates PGIol primer localisation on the segment of PGI sequence from Data Base. In that figure:

PGIol: - primer PGIol U (named in SGAP: BnPGIch 1 U)
 - primer PGIol L (named in SGAP: Bn PGIch 1 L)

5 PGIint: - primer PGIint U
 - primer PGIint L (is out side the sequence).

- Figure 5 illustrates electrophoresis gel of PGI-2 gene (PGIol), PCR marker and SG34, a PCR marker close to Rfo.

- Figure 6 illustrates Pgi-2 segment of DNA amplified by PCR with PGIol primers.

10 - Figure 7 illustrates digestion of the PCR product PGIol by MseI.

In that figure:

Sam and Darm has a 75bp band.

Drak, R211.Dk and R2000 showed a 70pb one (Acrylamide 15%).

8 was similar to Samourai (75bp) ; mix with Drakkar (70pb) it allowed the
 15 visualisation of the two bands.

- Figure 8 illustrates electrophoresis agarose gel of PGIUNT marker.

In that figure:

PGIUNT band (about 980bp) is present in B.oleracea, B.rapa cv Asko, maintainer and restored lines except in 'R211'.

20 There is no amplification in radish and Arabidopsis.

In various Brassica genotypes only one band was amplified. Size band are similar but sequences are different.

- Figure 9 illustrates electrophoresis gel of PGIint PCR marker.

In that figure PGIint of radish line 7 is of about 950bp. This band is the same as in
 25 the restored RRH1 and R113. It is not found in R211. It is not either in R2000.
 However the PGIint band is of a similar size of about 870bp in the various Brassica species, but sequences are different.

- Figure 10 illustrates electrophoresis agarose gel of Bo1Jon PCR marker.

- Figure 11 illustrates electrophoresis agarose gel of CP418 marker.

30 In that figure, the CP418 band (of about 670bp) is specific to the B.oleracea genome. It is present in B.ol, B.napus (Samourai, Drakkar, Pactol and the

heterozygous R2111*Dk). It is absent from the restored rapeseed (RRH, R113 and R211). It is present in the homozygous R2000.

- Figure 12 illustrates summary markers table.

- Figure 13 (13(a),13(b)) illustrates PGIol marker sequence alignment between
5 Arabidopsis, Radish, B.rapa, B.oleracea and R2000.

- Figure 14 (14(a), 14(b), 14(c), 14(d)) illustrates the PGIint-UNT marker sequence alignment between Arabidopsis, Radish, B.rapa, B.oleracea and R2000.

- Figure 15 (15(a), 15(b), 15(c)) illustrates the CP418L marker sequence alignment between Arabidopsis, Radish, B.rapa, B.oleracea and R2000.

10 - Figure 16 (16(a), 16(b), 16(c), 16(d), 16(e)) illustrates Arabidopsis, Radish and B.rapa BolJon markers. There are aligned with DB sequences of Arabidopsis (AC007190end-AC011000beginning), the B.oleracea EMBH959102 end and EMBH448336 beginning and representative consensus sequences of the SG129markers band 1 and 2 in B.napus (in Drakkar and Samourai respectively).

15 From the point 836bp, AC07190-AC11000 and GCPATpBOJ sequences are no longer closely homologous to the Brassica sequences.

The radish and B.rapa (GCPconsen RsRf BOJ and BR) sequences are still closely homologous to the B.napus one, from 858bp point to the 900bp and 981 points respectively.

20 In radish, only partial homology is found on the Brassica sequence further down.

In B.rapa species cv Asko, the left of its BolJon sequence can be aligned again, after a 78bp deletion, with those of B.oleracea and B.rapa in B. napus from the 1057bp point to the BolJon L primer.

- Figure 17 illustrates the localisation of Pgi-2 primers on the Arabidopsis th
25 MJB21.12 sequence.

- Figure 18 illustrates the BolJon primers localisation on the mipsAtl62850 gene and overlapping area of AC007190 and AC011000 Arabidopsis th clones. Alignment with the Arabidopsis BolJon PCR product (740bp) is presented.

It should be understood, however, that the examples are given solely by way of
30 illustration of the object of the invention, of which they in no way constitute a limitation.

Example I: method of producing a double low restorer line of *Brassica napus* for Ogura cytoplasmic male sterility (cms) presenting a radish introgression, carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

Materials and methods :

Genotypes: The 'R211' line with a deleted radish insertion was crossed to the spring low GLS rapeseed 'Drakkar' to produce a F1 progeny ('R211*Dk'). The spring low GLS cms line 'Wesroona' (australian origin) was used for following crosses. Were used as control in molecular analyses: Winter restored lines derived from 'Samourai' carrying the complete ('RRH1') or incomplete ('R113') introgression as well as European radish line7, Asiatic restored radish D81, hybrid *Brassica napus** wild radish, *Brassica oleracea*, and *B.rapa* cv Asko, *Arabidopsis thaliana*.

Gamma ray irradiation: Whole flowering plants were treated with gamma rays from a Co60 source in a controlled area. Sublethal dose fo 65 Gray was applied before meioses.

Testcrosses and F2 production: Irradiated plants were transferred in an insectproof greenhouse after removing flower buds larger than 2 mm. The irradiated F1 progeny was used to handpollinate the cms 'Wesroona' line. The restored derived F1' plants were allowed to produce F2 families harvested individually and precisely sown in a field assay along with non irradiated controls (Fig 1).

Phenotypic selection: Three visual criteria were scored (on a 1 to 5 scale) over 2 years in field assays, on 1200 F2 offsprings plus 44 controls (82 330 quoted plants):

- 1-Vegetative vigour,
- 2- Normality of the ratio of fertile /sterile plants in the F2 segregation, and
- 3- Female fertility (pod development and seed set).

Advanced selfed generations of the selected families were obtained either in field or greenhouse and produced homozygous lines (F4) for further analysis.

Isozyme analysis was performed as in (Delourme R. and Eber F. 1992. *Theor Appl Genet* 85: 222-228), marker development from (Fourmann M et al 2002.

Theor Appl. Genet. 105:1196-1206.): PCR products are validated by sequencing. Alignments were made using Blast Ncbi and Uk Crop Net Brassica DB and the Multialin software INRA Toulouse.

Method :

- 5 We choose one low GLS spring homozygous restorer line, 'R211', already exhibiting deletions in the introgression (Delourme R. and Eber F. 1992. *Theor Appl Genet* 85: 222-228. Delourme R et al 1998. *Theor Appl Genet* 97: 129-134. Delourme R. et al 1999. *10th Int. Rapeseed Congress, Canberra*). Several molecular markers are missing on either side of Rfo, such as spATCH1A (Fourmann M et al
10 2002. *Theor Appl. Genet.* 105:1196-1206); spSG91 (Giancola S et al 2003 *Theor Appl. Genet.* (in press)). 'R211' lost the isozyme expression of the Pgi-2 allele of the radish gene but also the one of Pgi-2 allele of B.oleracea genome (1,2). Moreover, the homozygous 'R211' shows linked negative traits such as low vigour and very poor seed set. We hypothesised that these plant lack a rapeseed
15 chromosomal segment. The fertile ratio in F2 progenies derived from this material is lower than expected (64% instead of 75%). We initiated the program from this 'R211' line and tried to force recombination between the Rfo carrying introgression from this deleted line and the rapeseed homologous chromosome from a double low B. napus line.
- 20 Ionising irradiation is known to induce chromosomal rearrangements by double strand breaks followed by aberrant rejoining of the ends. Gamma-ray irradiation was used on a heterozygous F1 derived from the 'R211' line to induce chromosome breaks, just before meiosis, aiming at a recombination of the deleted radish introgression in the rapeseed genome.

25 Results :

Very few families were at the best score for the three criteria out of 1200 F2 families tested.

- Only one, 'R2000', proved to produce a normal ratio of fertile plants per selfed progeny with a stable recovery of good agronomic traits such as a good female
30 fertility, with a normal seed set compared to 'R211' (Fig 2 and 3). This family was obtained from a 6 mn irradiation treatment at a dose flow of 65 Gray per hour. Glucosinolate analysis confirmed its low content.

In figure 2 (Seed set on 'R211' and 'R2000') R2000 showed normal inflorescences, with a normal looking architecture.

In figure 3 (Number of seeds per pod), we observe:

- on the best 'R2000' F4 families in self pollination (Selfings) and in testcrosses
- 5 - on 'Pactol' cms line on rapeseed and 'R211' controls.

Example II: selection of markers in the Pgi-2 gene

PGI isoenzyme analysis: 'R2000' progeny expressed the rapeseed Pgi-2 allele from *B. oleracea* genome, originally lost in 'R211'.

- 10 Three PCR markers were defined to characterise the R2000 family compared to the known restorer rapeseed RRH1 and R113.

1) PGIol marker was developed from the BrassicaDB sequences to be specific to the Brassica genome. There is no amplification in radish nor in *Arabidopsis th.*, but only in Brassica, with one 248 bp band.

- 15 2) PGIint marker amplified a longer part of the Pgi-2 gene, allowing clear distinction between the various tested species Brassica, *Raphanus* and *Arabidopsis*. The species *B.rapa* and *B.oleracea* were not distinguished by the band size on agarose gel, but by their PGINT band sequence.

- 3) PGIUnt marker, a combination of the PGI ol U and PGI int L primers.
- 20 This marker had the specificity of the PGIol marker but amplifying a longer part as for PGIint one.

II.1 PGIol marker

- With the PGIol primers, the 'R211' parental line showed no amplification, while the spring tested lines showed a 248bp band. Its DNA sequence is homologous to
- 25 the PGI-2 sequences from the Crop Net UK DB in Brassica species and from previous work in our group (named SGAP sequences) (Localisation of the primers SG PGI chou, Fig 4).

It was ortholog of the clone MJB21-12, on the chromosome V, (34543bp) in *Arabidopsis* (NCBI DB).

- 30 PGIol plus SG34 to set an Homozygosity test:

The combined use of two sets of primers in a mix PCR, PGIol marking the Pgi-2 gene absent in the homozygote restored plant and SG34 (from S. Giancola et al,

Giancola S et al 2003 *Theor Appl. Genet. (in press)*), a very close marker to the Rfo gene, was set up to discriminate homozygous from heterozygous plant among the fertile plants segregating in F2 progenies derived from 'R211'. In place of using SG34, it is possible to use any other marker close to or in the Rfo gene.

- 5 Only one family R2000 showed no difference between homozygote and heterozygote offsprings:

The Pgi-2 gene is present in the R2000 homozygote, which is not the case for the parental homozygous R211.

In figure 5 (PGIol and SG34 PCR markers):

- 10 The homozygous 'R2000' family has recovered the PGIol band.

DNA sequence of the band confirmed the homology with the known Arabidopsis and Brassica Pgi-2 sequence. Control genotypes (Drakkar, Pactol, and, Samourai, Darmor) had the same pattern on the gel. Sequence of this common band allowed to confirm their high homology as they were quasi similar except one base substitution.

- 15

The homozygous 'R2000' family has recovered the PGIol band of the Brassica oleracea type. It was distinct from the known restorer of the Samourai group.

This amplified part of the Pgi-2 is very conserved and hardly any differences were shown among the various genotypes. A longer part of Pgi-2 gene was investigated.

- 20 II.2 PGIUNT and PGIint markers

Electrophoresis Patterns of PCR products:

PGIUNT marker: A second reverse primer, PGIint L, was designed further down the Pgi-2 sequence, to amplify as well conserved and as variable regions of the gene. When used with the PGIol U primer, it amplifies a 980bp band only in Brassica

- 25 genomes.

R211 didn't show any band, The homozygous 'R2000' showed the PGIUNT band as in the Drakkar parent.

In figure 8 (PGIUNT marker):

- 30 PGIint marker amplified a segment of PGIUNT. The upper primer PGIint allows the amplification in all tested species, allowing a clear distinction between Arabidopsis, Radish and Brassica. B.rapa and B.oleracea were not distinguished by the band size on agarose gel, but by their PGIint sequence. All tested restored genotypes, but the

'R211' line, exhibited the European radish band and one Brassica band, homologous to the B.rapa one.

The homozygous 'R2000' didn't show the radish PGIint band, as in the deleted 'R211' parental line, but showed one Brassica band, homologous to the B. oleracea one.

Electrophoresis of PGIint marker is represented in figure 9.

Sequence analysis:

Comparison of the PGI sequences from the data bases.

A PGI segment of about 490bp is known.

- 10 Sequences of a segment of about 490bp from different genotypes (B. oleracea, B. rapa, B. napus) have been studied in our laboratory group and some sequences were given to Brassica Crop Net DB: EMAF25875 to 25788 by M.Fouramni (4) These sequences are very conserved.

Comparison of the B. rapa et B.oleracea species PGI sequences (figures 13 and 14):

- 15 Comparison between PGI sequences we have obtained from the tested genotypes of B.oleracea and B.rapa species, showed that they were distinct by 21 base substitutions. Theses substitutions allowed to distinguish PGIint sequences from the other tested genotypes of rapeseed, homologous to either B.rapa cv Asko (RRH1 and R113) or B.oleracea (Drakkar, R211*DK but also R2000).

20

Example III: selection of marker in a region close to Rfo

Markers surrounding the Rfo gene in the radish insertion were determined in order to facilitate the Rfo gene cloning (Desloires S et al 2003 *EMBO reports* 4, 6:588-594). One of these, the SG129 PCR marker was located very close to Rfo

25 (Giancola S et al 2003 *Theor Appl. Genet. (in press)*): it co-amplified distinct bands in B.oleracea and B.rapa genomes of B.napus, but the radish band was very difficult to see on an agarose gel.

- The target SG129 sequence was ortholog of a clone (AC011000, at the locus F16P17) in *Arabidopsis thaliana*. This clone overlapped an *Arabidopsis* adjacent
- 30 contig clone (AC07190).

From the Brassica Crop Net DB, we found one B.oleracea clone, (EMBH448336, 764bp) blasting with the beginning of the A011000, and a second B.oleracea clone

(EMB53971), distant from about 300bp on the Arabidopsis map, that blasted with the end of ACO7190.

We designed a new PCR marker, BolJon, between the two B.oleracea clones. We verified that it allowed amplification of a specific PCR bands in the different
5 genotypes compared here.

In figure 16 (electrophoresis gel of BolJon PCR products):

- In Arabidopsis, a BolJon 815bp band was amplified, homologue to the overlapping segment of the contigs.
- In Brassiceae diploid species, BolJon marker showed distinct bands: one of
10 950bp in B.oleracea and one of 870bp in B.rapa. It showed that the two B.oleracea clones (EMB53971 and EMB448336) are in sequence continuity in Brassica genome as it is for the ortholog sequences in Arabidopsis.
- In B.napus, these two bands are co-amplified in the maintainer lines, Samourai or Drakkar.
- 15 - In radish line7, one BolJon band was amplified of about 630 bp long. The band of the restored radish cmsRd81 was slightly smaller.
- In all the restored rapeseed lines, one of the BolJon bands was of the same size as the radish line7. BolJon is a marker of the radish introgression.
- The homozygous restored rapeseed lines, 'RRH1', 'R113' and also 'R211',
20 only showed the B.rapa band and the 630bp radish band bp suggesting the B.oleracea ortholog of the target gene is absent or has been modified when the radish segment of chromosome was inserted into the rapeseed B.oleracea constitutive genome.

'R2000' homozygote plants showed radish PCR BolJon, plus the two Brassica
25 BolJon bands, again having recovered the B.oleracea one, lost in 'R211' and other restorer lines.

We designed a primer, pCP418L, specific of the B.oleracea genome in the tested species. With the SG129U primer it amplified only one PCR band (670bp) in the B.oleracea species. (Fig 17)

30 There was no amplification in B.rapa, in radish, nor in Arabidopsis, but there was a clear CP418 band in B. napus maintainer lines. Its sequence was strictly homologous to the EMB448336 sequence. This marker was in a very conserved

DNA sequence allowing no polymorphism between genotypes except by presence / absence.

In RRH1, R113 and in R211 there was no CP418 band, indicating as previously that the *B.oleracea* ortholog of the target gene is absent or has been modified following
5 the radish insertion.

'R2000' homozygote plants showed CP418 band, again having recovered the specific *B.oleracea* one.

In the present invention, a new recombined low GLS restorer line has been selected with a good female fertility. The poor value of line 'R211' allowed selection in the
10 field for a rare recombination event and characterisation the 'R2000' family.

The homozygous 'R2000' presents a unique combination of the PGIol, PGIUNT, PGIint and BolJon markers when compared with the rapeseed restorer analysed yet: PGIint marker showed that the homozygous restored rapeseed lines, RRH1 and R113 presented the European radish band plus one Brassica band, homologous to
15 *B.rapa* genome. 'R2000' shows no radish band, lost as in its parental deleted line R211, but showed one Brassica band homologous to *B.oleracea*. The ortholog PGIint sequence in its *B.rapa* genome is not amplified with this marker in R211 and Drakkar genetic background.

PGIol marker and PGIUNT marker sequences in restored lines RRH1 and R113
20 were homologous to the *B.rapa* cv Asko one. In 'R2000', PGIUNT sequence is homologous to *B.oleracea*. The ortholog PGIunt sequence in its *B.rapa* genome is not amplified with this marker in R211 and Drakkar genetic background.

BolJon marker showed that the homozygous restored rapeseed lines, including 'R211' presented the European radish band plus only the *B.rapa* one. 'R2000'
25 shows the two bands of 'R211' plus the recovered *B.oleracea* BolJon band.

CP418 marker showed that 'R2000' recovered this conserved *B.oleracea* segment.

Our hypothesis is that a recombination event took place in the pollen mother cell which gave rise to 'R2000' plants. The deleted radish introgression was then integrated to the normal homologous chromosome segment, carrying the *B.oleracea*
30 type Pgi-2 gene and BolJon target sequence, characterised by these markers, probably from the Drakkar '00' genome present in the irradiated heterozygous 'R211*DK'.

The pattern observed for BolJon suggests that the recombination event resulted in a particular duplicated region, one from radish and one *B.oleracea*, in the 'R2000' family.

CLAIMS

1. A method of producing double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, said method including the step of:
 - a) crossing double low cms lines of spring Brassica napus comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of Brassica napus,
 - b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation,
 - c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line,
 - d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,
 - e) selecting progeny lines.
2. A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.
3. A method according to claim 1 wherein the double low cms line of spring Brassica napus of step a) is R211.
4. A method according to claim 1 wherein the testing is performed with the combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.
5. Double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a

good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

- 5 6. Double low restorer lines of Brassica napus according to claim 5, wherein they present a unique combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.
7. Brassica napus hybrid plants and progeny thereof obtained through the steps of:
 - 10 a) providing a restorer line produced according to claim 1 and bred to be homozygous,
 - b) using said restorer line in a hybrid production field as the pollinator,
 - c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant, and
 - 15 d) harvesting the hybrid seed from the male sterile plant.
8. The seeds of Brassica plant developed from the Brassica line obtained in claim 1.
- 20 9. The seeds of Brassica napus obtained in claim 7.
10. The seeds of Brassica napus obtained in claims 1 and 2 deposited in NCIMB Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on July 4 , 2003, under the reference number NCIMB41183.
- 25 11. Use of the combination of at least four markers PGIol, PGIint, BolJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombined restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined
 - 30 with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

12. Use according to claim 11 wherein the combination is of five markers PGIol, PGIUNT, PGIint, BolJon and CP418.

5 13. Use according to claim 12, wherein:

- The marker PGIol is amplified using the primers: PGIol U and PGIol L
(PGIol U: 5'TCATTGATTGTTGCGCCTG3';

PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')

- The marker PGIint is amplified using the primers: PGIint U and PGIint L
(PGIint U: 5'CAGCACTAATCTTGCGGTATG3';

PGIint L: 5'CAATAACCCTAAAAGCACCTG3')

- The marker PGIUNT is amplified using the primers: PGIol U and PGIint L:
(PGIol U: 5'TCATTGATTGTTGCGCCTG3';

PGIint L: 5'CAATAACCCTAAAAGCACCTG3')

- The marker BolJon is amplified using the primers: BolJon U and BolJon L:
(BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';

BolJon L: 5'GCCTACTCCTCAAATCACTCT3')

- The marker CP418 is amplified using the primers: SG129 U and pCP418 L:
(SG129 U: cf Giancola et al (5)

pCP418 L: 5'AATTTCTCCATCACAAGGACC3')

14. PGIol marker whose sequence follows:

25 TCATTGATT GTTGC GCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60
ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120
AATCTTGCGG TATGAATTTG TGATTAAATT TGTTTGTTTG TGAATCTTTC TTCATTGTTT 180
GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTACAAA CTGAATTTTC TACCGGGTCT 240
GATGTACA 248

15. PGIUNT marker whose sequence follows:

30 TCATTGATT GTTGC GCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60
ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120
AATCTTGCGG TATGAATTTG TGATTAAATT TGTTTGTTTG TGAATCTTTC TTCATTGTTT 180
GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTACAAA TGAATTTTCT ACCGGGTCTG 240
ATGTACAATG CTAGTCTCCA TGTTCTTGGG GATCATGATT TATTTTCTAC ATGTATTTCAG 300
35 ACAGTACAGA AGAAAGTGTT CAAAACTCTG GATGTTTTAA TTTACAGTTA GTGGAGAAGT 360
TCGGCATTGA TCCGAACAAAT GCATTTCAT TTTGGGACTG GGTGGGTGGA AGGTACAGTG 420
GTAAGTGCTT GTTTATTTGG TTGTATAAAT TTCTCGTCCA TTTCCGCTTG CTTAGTGTAT 480
AACTGAAATT CTTTTCAGT TTGCAGTGCT GTTGGAGTCT TACCATTGTC TCTACAGTAT 540
GGCTTCTCTG TGGTTGAGAA GTACGGTACC TTCTACTTTA TCAGCCATCT CATAAAATGT 600

CTTAGGCATA TTCTTTCTAT TTTATTTCCC TCTTAATGAT TTCTTCTTTT TTTTATTGCA 660
 TTCCCGTTTT ATTTTCAAAA GTTGTTACTG TCTCTAAATC AAGAAGAAAC CTTCTTAGTA 720
 GATCCAGCTG ATATTCAGCC TTTTAAAT TGGACTGCAG GTTTTAAAG GGGAGCTTCA 780
 AGCATTGATA AGCATTTCCT GTCCACACCG TTTGAGAAGA ATATACCCGT GAGTTGCATT 840
 5 AGTTGTGTGA TTATACAGTT TTCTTGTCCT TTTGCTATGT CCATCAACAC TAGAGATTCTG 900
 TGAAGTTATT AGTGTAGTCA ACGCATAGGG AGAGGTGATT GGTGACTTTT GGACGATTTT 960
 AGGTGCTTTA GGGTTATTG 979

16. PGInt marker whose sequence follows:

10 CAGCACTAAT CTTGCGGTAT GAATTTGTGA TTAAATTTGT TTGTTTGTGA CTCTTTCTTC 60
 ATTGTTCTGT TCTGTACAAT AAACCGAATG TATAATCTTT TACAAACTGA ATTTTCTACC 120
 GGGTCTGATG TACAATGCTA GTCTCCATGT TCTTGGGGAT CATGATTTAT TTTCTACATG 180
 TATTCAGACA GTACAGAAGA AAGTGTTCAA AACTCTGGAT GTTTTAATTT ACAGTTAGTG 240
 GAGAAGTTCT GCATTGATCC GAACAATGCA TTTGCATTTT GGGACTGGGT TGGTGGGAAGG 300
 15 TACAGTGGTA AGTGCTTGTT TATTTGGTTG TATAAATTTT TCGTCCATTT CCGCTTGCTT 360
 AGTGATAAAC TGAAATTCTT TTGCAGTTTG CAGTGCTGTT GGAGTCTTAC CATTGTCTCT 420
 ACAGTAGGGC TTCTCTGTGG TTGAGAAGTA CGGTACCTTC TACTTTATCA GCCATCTCAT 480
 AAAATGTCTT AGGCATATTC TTTCTATTTT ATTTCCCTCT TAATGATTTT TTCTTTTTTT 540
 TATTGCATTC CCGTTTTATT TTCAAAAGTT GTTACTGTCT CTAAATCAAG AAGAAACCTT 600
 20 CTTAGTAGAT CCAGCTGATA TTCAGCCTTT TTTAAATTGG ACTGCAGGTT TTTAAAGGGG 660
 AGCTTCAAGC ATTGATAAGC ATTTCCAGTC CACACCGTTT GAGAAGAATA TACCCGTGAG 720
 TTGCATTAGT TGTGTGATTA TACAGTTTTC TTGTCTTTT GCTATGTCCA TCAACACTAG 780
 AGATTCTGTA AGTTATTAGT GTAGTCAACG CATAGGGAGA GGTGATTGGT GACTTTTGGG 840
 CGATTCAGG TGCTTTAGGG TTATTG 866
 25

17. BolJon marker whose sequence follows:

GATCCGATTC TTCTCCTGTT GAGATCAGCT CCAAACATCA AACAACTTGT ACACAAATAT 60
 CTTTACTTGC TAAATGGAAC ATGACAAGAG ATAGAAAATC TTGCTCATAG TATTGTACAA 120
 GGGATAACAG TGTAGAAAAC AAACCGTCTG TAAGATTTTC TCCCTGATCC TCTCACTTAA 180
 30 CCAGTAGGCG TTTTTCACAT TGAAGCGCAT ATCTACTTTG GTATTCAGT AATAAAAAAA 240
 GAAAGCTGGT AACATGTGAA GGATATACAA GCATTGATAC ACCAAGTAGT CACAAACTAC 300
 ATTATAAAGG TCAGACCTTT GTTCACATTC TGGCCTCCAG GACCACCGCT TCTAGCAAAG 360
 TTAAGCGTAA CATGGTCTGC ACGTATACAA ATGAAAATGT TTCTATCAAA ATCCTATAAA 420
 ATAGAGCTCT ATAACATTGT CGATACATAG TTTCACTAAC TCTGCAAGTA CTAAACACAT 480
 35 ATACAAACAA AACTATGCGA ACAGATCAAA ACTACTACAG AACACAGTTC TATGACACTG 540
 TCGATAGTAA CATCCTCTGC AAGTACCAA GAGATAGCAA ATGAAACTAT GTAAACAAAT 600
 CAAAATTCTA AATTTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTTCT 660
 GTAAATATTT CCATCAAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGTAAATGT 720
 TCCAACAAA CCACTACATA GCAGAGTTCT TATAACATTG TCTGTAAATG TCCAATCAAA 780
 40 ACCACTACAG AACAAAGCTC CTATAACATT GTTTATACAA AGTTTCACTA AATCTACAAA 840
 CTTTCCCCGT AAATGAGCTT AATATCACCC AAAGATGTTT CAATCAGATA AAGAGTACGA 900
 CATCGTTTTG AGATTAGAAC AAATGAAAC TTACGTAGAG TGATTTGAGG AGTAGGC 957

18. CP418 marker whose sequence follows:

45 AATTTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTTCT GTAAATATTT 60
 CCATCAAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGTAAATGT TCCAACAAA 120
 CCACTACATA GCAGAGTTCT TATAACATTG TCTGTAAATG TCCAATCAAA ACCACTACAG 180
 AACAAAGCTC CTATAACATT GTTTATACAA AGTTTCACTA AATCTACAAA CTTTCCCCGT 240
 AAATGAGCTT AATATCACCC AAAGATGTTT CAATCAGATA AAGAGTAAAC ACATCGTTTT 300
 50 GAGATTAGAA CAAACTGAAA CTTACGTAGA GTGATTTGAG GAGTAGGCTC GTTGCCAGCA 360
 GAGCTAGCTC TCTCCTCCGC CTCATGAAGC ATCTGTTGCA CCTGAGACAA CCGTGACGAA 420
 ACTTTCCGAT CACCGCCACC AGAATTCGAC GCCGCGCATC GGAAGGATCC GAATCGGGAA 480
 CTGAGTGAAC CCGAGCGATC CCGGAGTGC GACGGAGCGA TGGGAAAAGA GAGTGGCAGC 540
 ATTTGACGCA AGAGTGGGAG AGGAGAGGGT GGTGGATAAA CTCGCGTATG ATCAAGTTTCG 600
 55 TCATCGTCTT GATTGCCGCC ATTTTTTTTG TCAGGCGCT CTGTGGCTTA GAAGTTTCCG 660
 atgtcaatga ac 672

Method of producing double low restorer lines of Brassica napus
having a good agronomic value

ABSTRACT

A method of producing double low restorer line of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterized by female fertility, a good transmission rate of Rfo and a high vegetative vigour. A method of forming Brassica napus hybrid seeds and progeny thereof. The seeds of Brassica napus and use of the combined markers PGIol, PGIunt, PGIint, BolJon and CP418 for characterising.

Fig 1

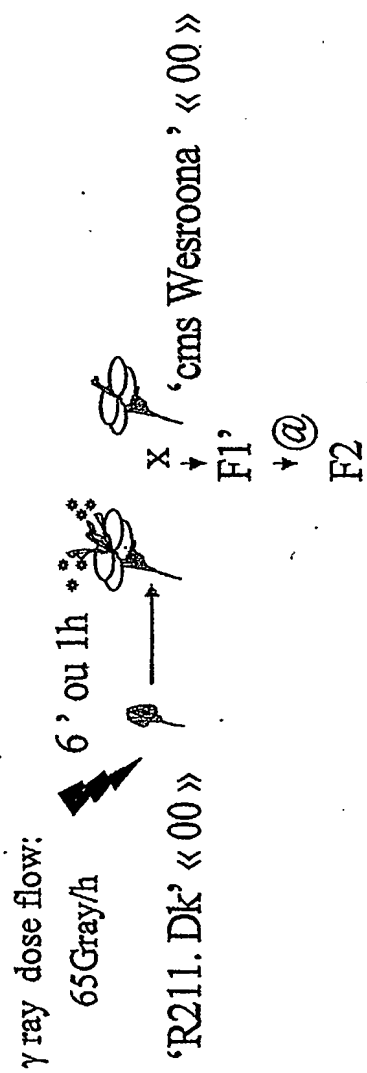


Fig 2

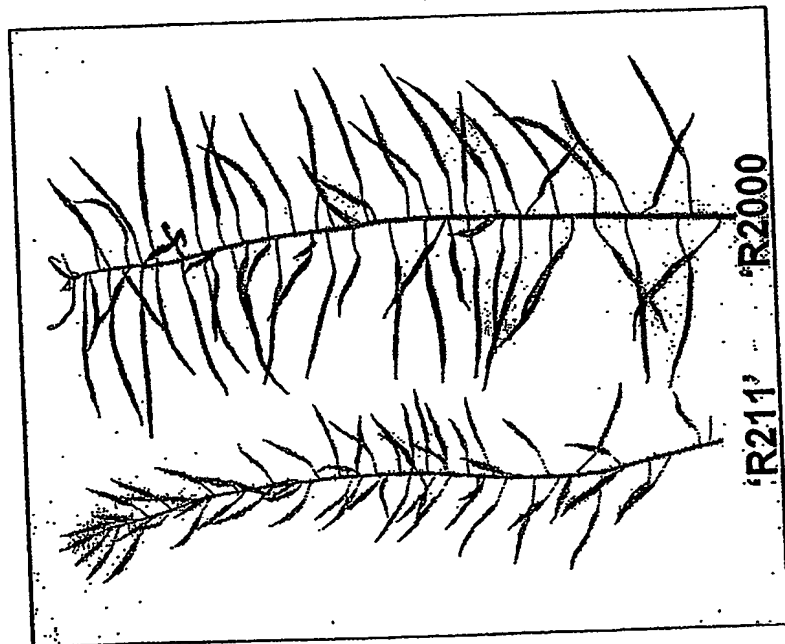


Fig 3

Genotype	Selfings	Test Crosses
Drakkar	29.3	
Pactol	23.1	
R211	11.2	25.5
R2000	26.5 (24.0 – 31.1)	27.0 (24.0 – 28.7)

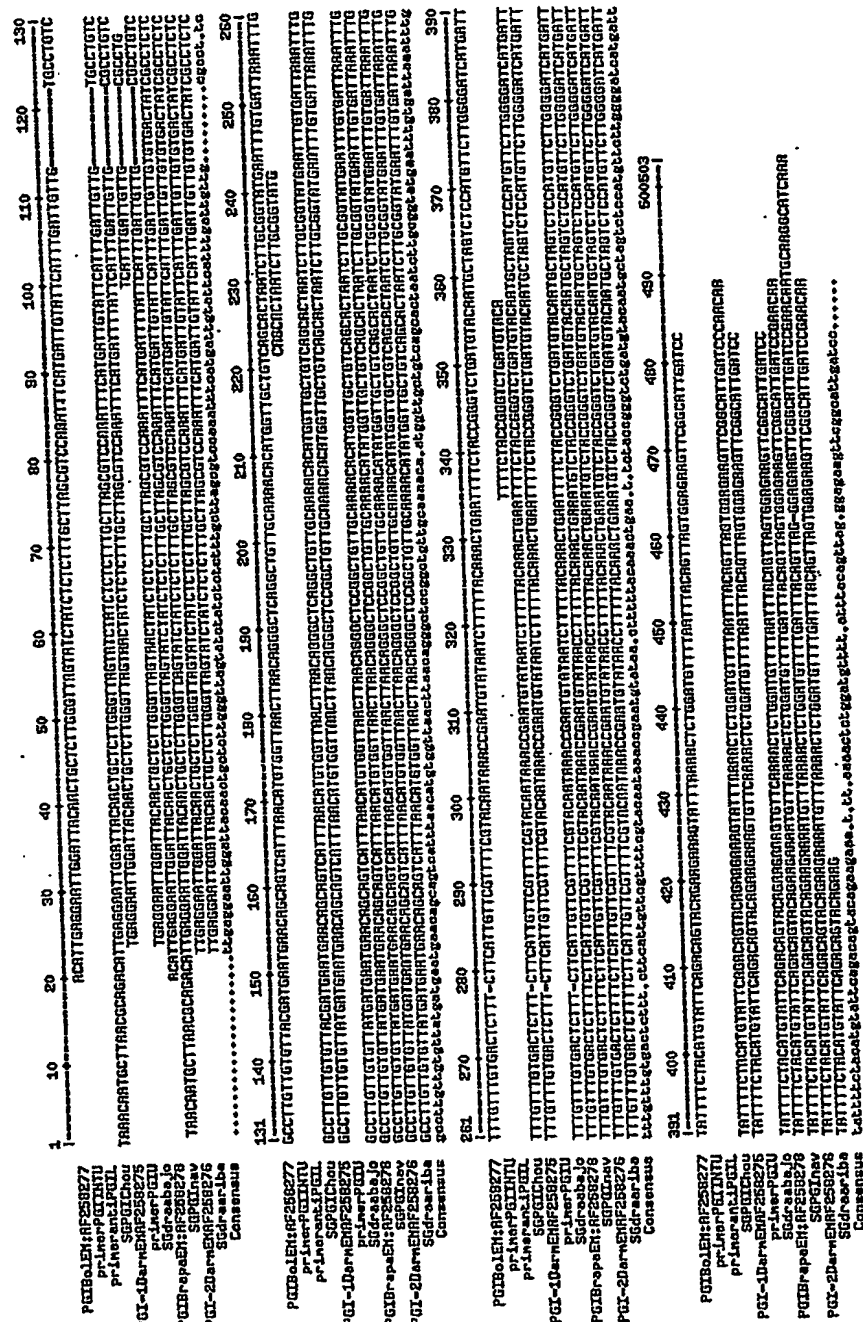
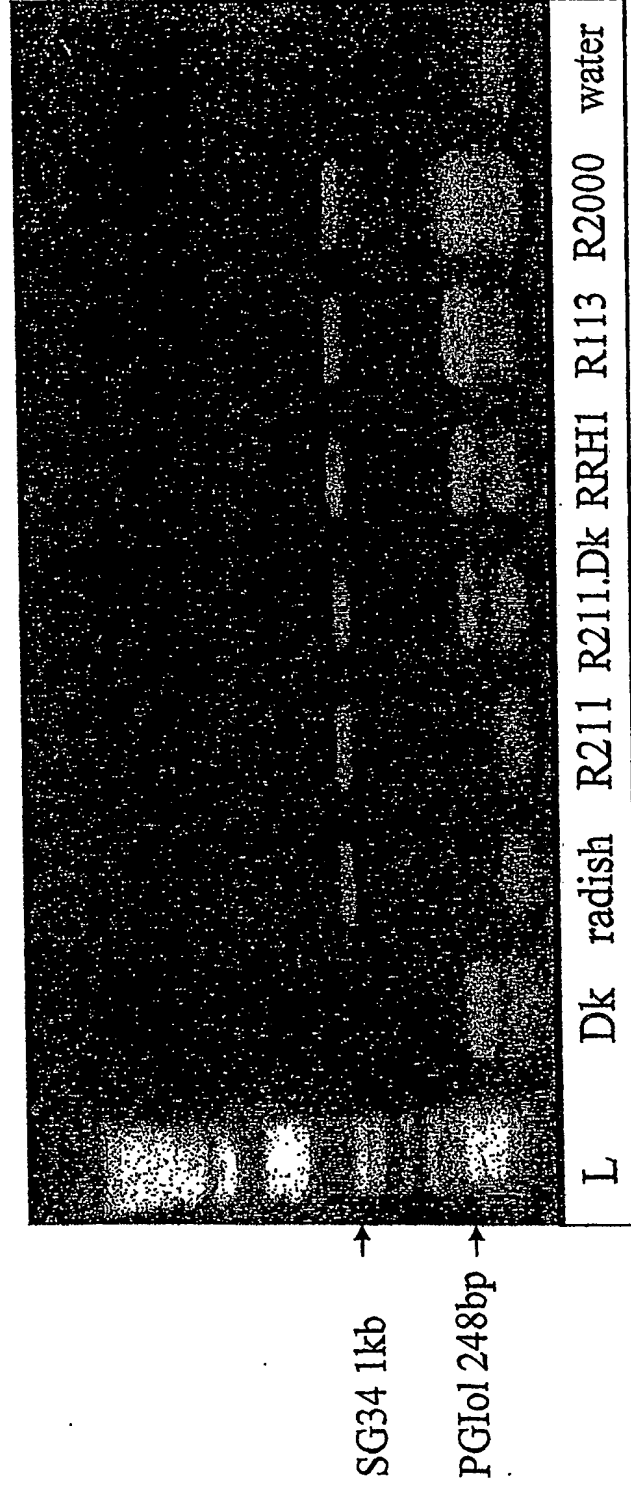
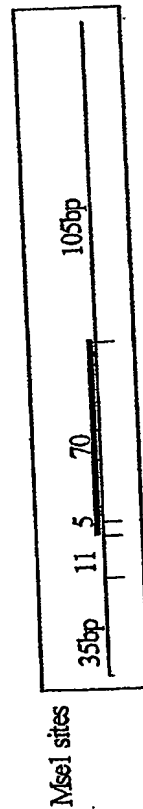


Fig 5

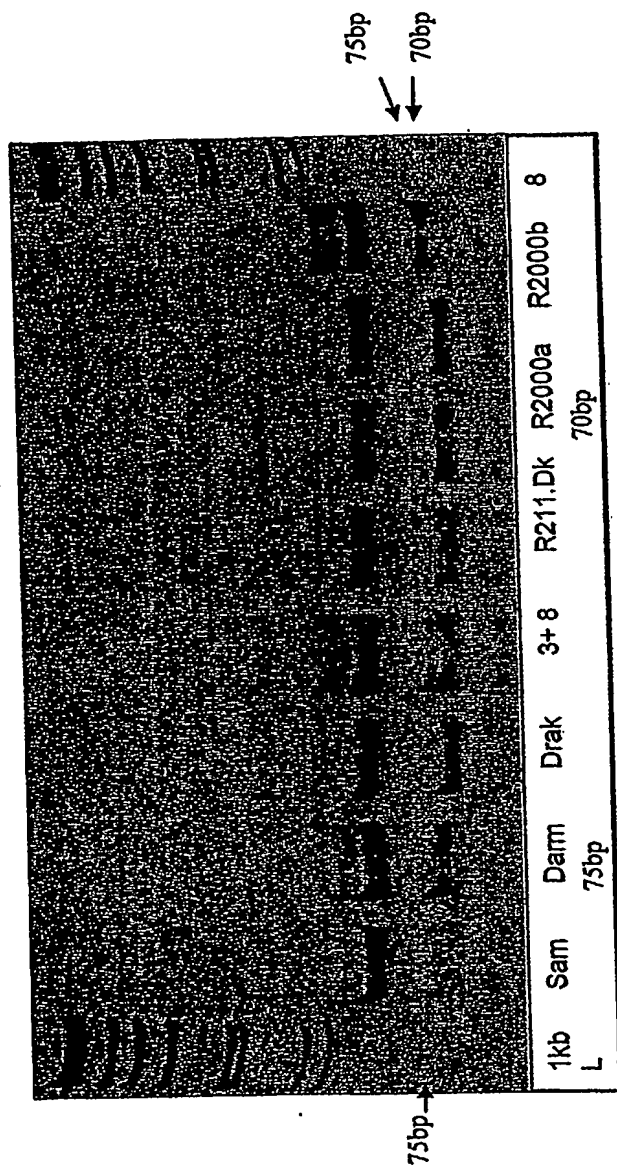




T in Drakkar and R2000

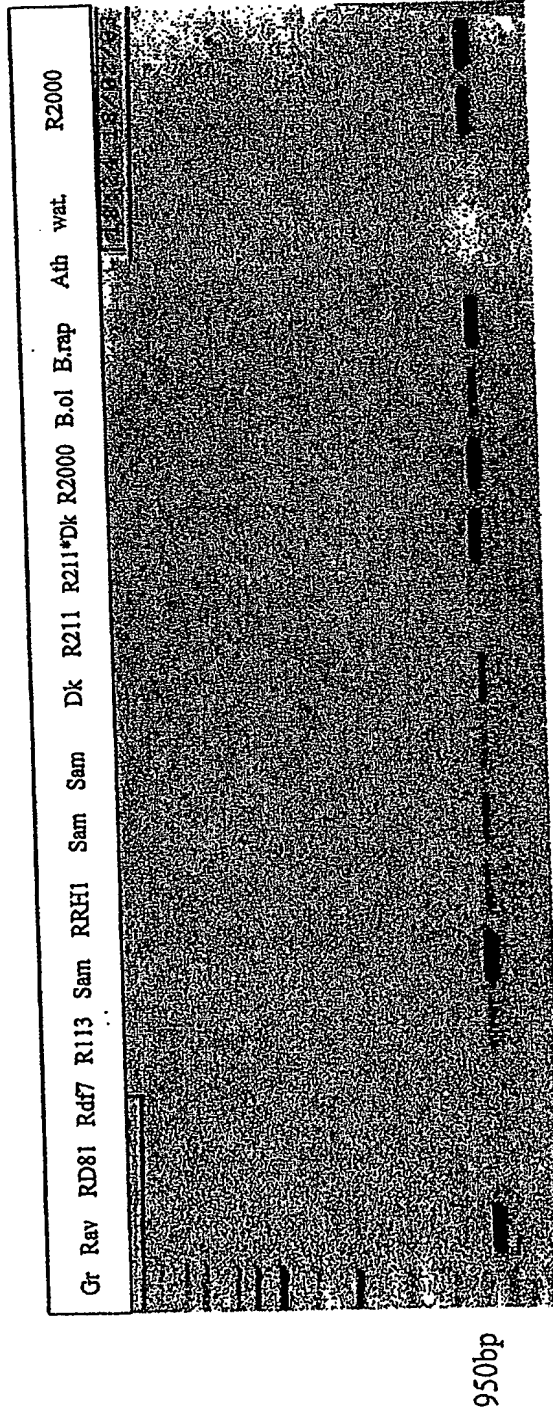
MseI restriction enzyme cut DNA sequences at the T/TAA sites (*)

Fig 7



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Fig 8



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Fig 9.

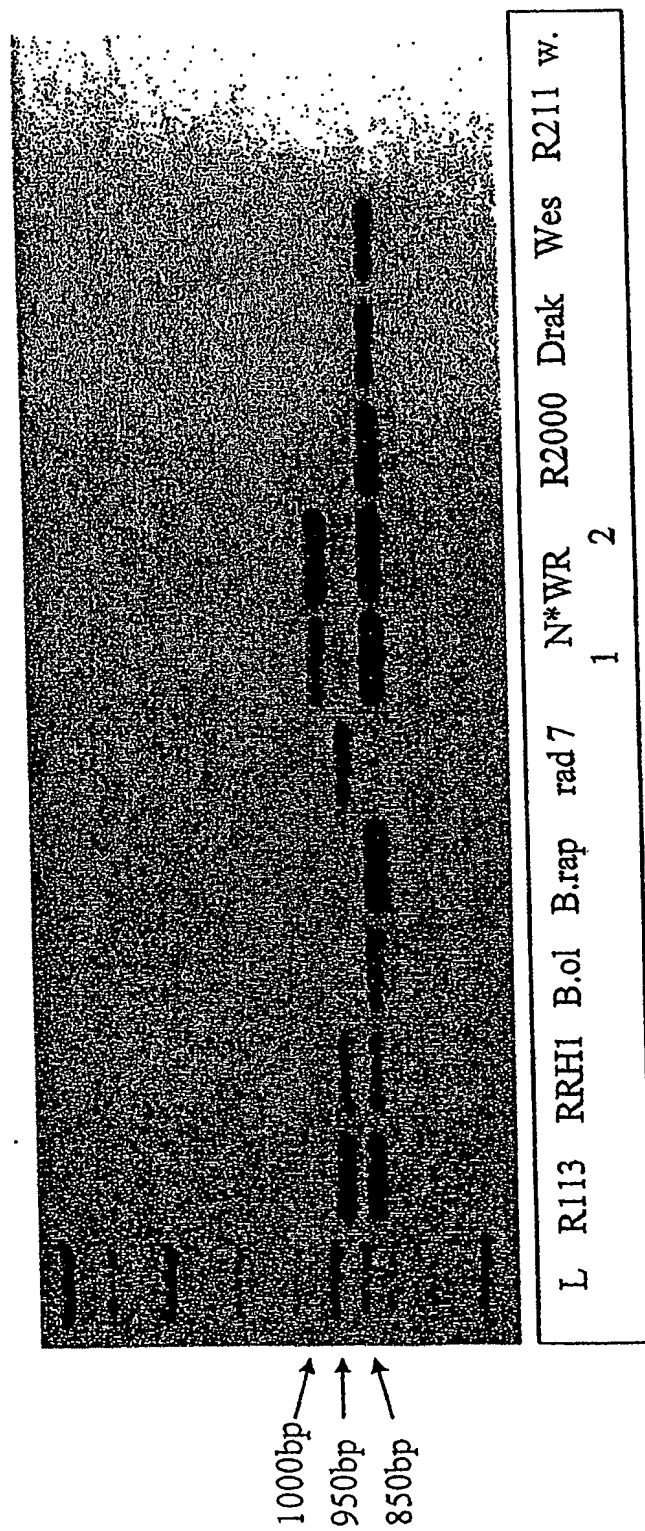
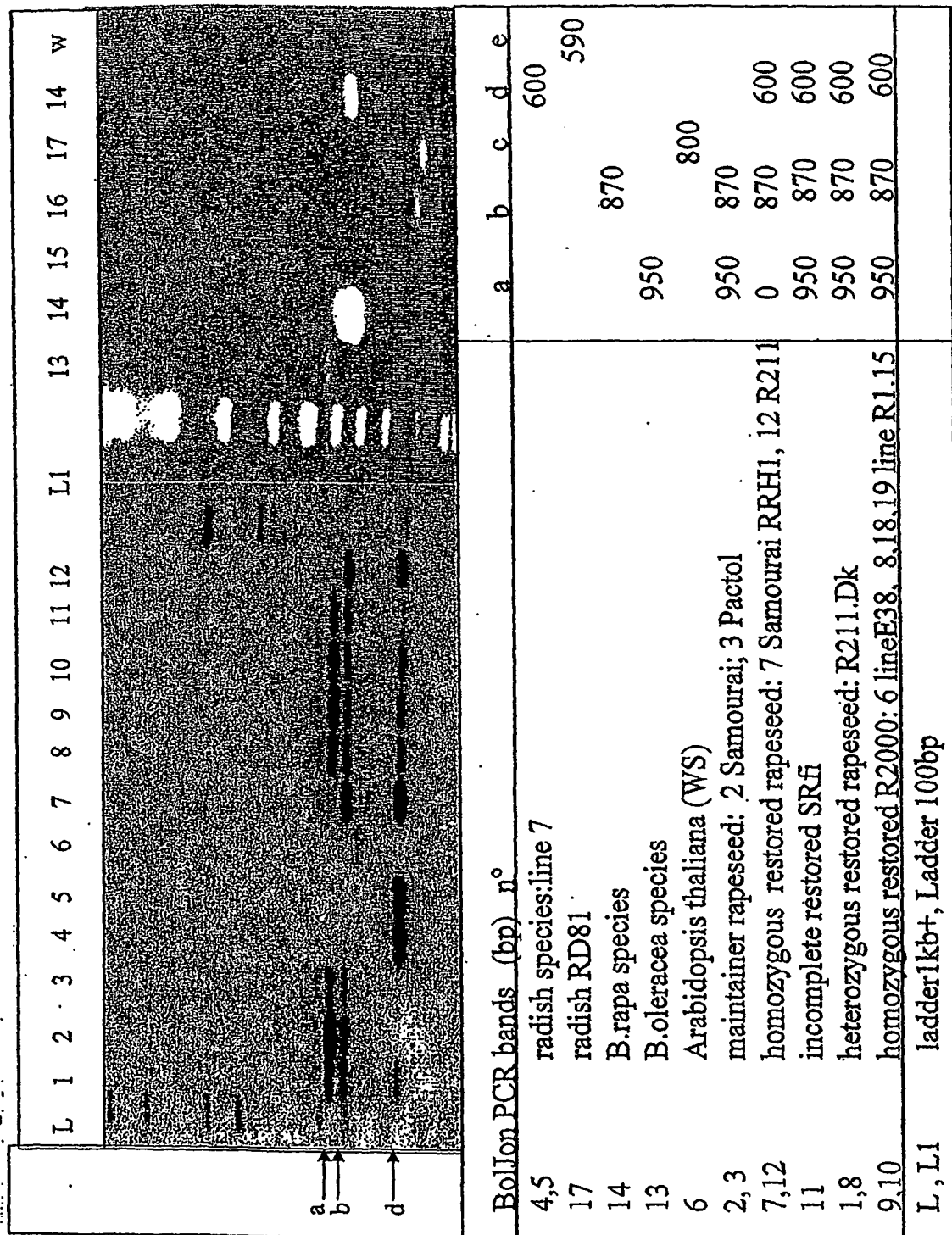
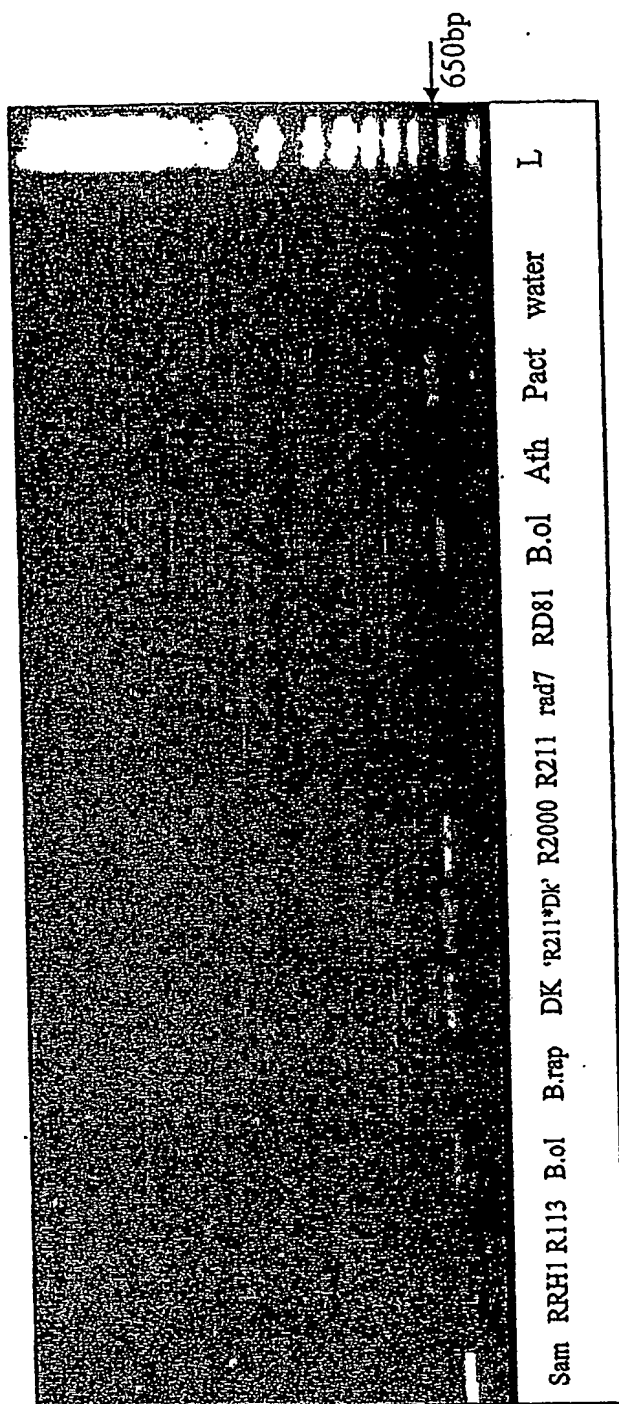


Fig. 10



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Fig 11



The CP418 band (about 650bp) specific to the *B. oleracea* genome.

It is present in *B. ol.*, *B. napus* (Samourai, Drakkar, Pactol and the heterozygous R211*Dk)

It is absent from the restored rapeseed (RRH, R113 and R211)

It is present in the homozygous R2000.

Figure 12

[illegible]

Figure 13 (a)

	51		81 PGIol U --->	100
consePGIintUNTDrakka
consensWesrPGI
consePGIintUNTR113
consePGIintUNTBrapaA
ConsePGIintUNTRRH1
PGIBo-EM:AF258277	TTGCTTAGCG	TCCAAATTTC	ATGATTGTAT	TCATTTGATT GTTGTG....
PGIBra-EM:AF258278	TTGCTTAGCG	TCCAAATTTC	ATGATTGTAT	TCATTTGATT GTTGTGTGAC
consePGIintUNTBolera
consePGIintUNTR2000TTG...	TCATT.GA...	TTGT.TGCG
Consensus				1
	101	====>		150
consePGIintUNTDrakkaGTCG	TTTGTGGTG	AGT.GAACAG CAGTCATTTA
consensWesrPGIGCCTGTTT	TGTTATGATG	AAT.GAACAG CAGTCATTTA
consePGIintUNTR113GCCGGTTGG	TAT.GAAACG CAG.CATTTA
consePGIintUNTBrapaAG	CAGTCATTTA
ConsePGIintUNTRRH1CG	TGTTGAGAAG CAG.CATTTA
PGIBo-EM:AF258277	CCTG	TCGCCTTGTTG	TGTTA.GATG AAT.GAACAG CAGTCATTTA
PGIBra-EM:AF258278	TATCGCCTC	TCGCCTTGTTG	TGTTATGATG AAT.GAACAG CAGTCATTTA
consePGIintUNTBolera
consePGIintUNTR2000	CCTG	TCGCCTTGTTG	TGTTATGATG AAT.GAACAG CAGTCATTTA
Consensust.gg ...t.gaa.ag cagtcattta
	151		* MseI restriction site	200
consePGIintUNTDrakka	ACATG.TGGT	TAACCTTAACA	GGGCTCCGGC	TGTTGCAAAA CACATGGTTG
consensWesrPGI	ACATG.TGGT	TAACCTTAACA	GGGCTCCGGC	TGTTGCAAAA CACATGGTTG
consePGIintUNTR113	ACATG.TGGT	.AACTGAACA	GGGCTCCGGC	TGTTGCCC.. CTAAGGGTTG
consePGIintUNTBrapaA	ACATGGTGGT	TAACCTGAACA	GGGCTCCGGC	TGTTGCAAAA CACATGGTTG
ConsePGIintUNTRRH1	ACATG..GGT	.AACTGAACA	GGGC.CCGGC	TGTTGCAA.. .ACAG...TG
PGIBo-EM:AF258277	ACATG.TGGT	TAACCTTAACA	GGGCTCAGGC	TGTTGCAAAA CACATGGTTG
PGIBra-EM:AF258278	ACATG.TGGT	TAACCTTAACA	GGGCTCCGGC	TGTTGCAAAA CATATGGTTG
consePGIintUNTBoleraC	TGTTGCAAAA CACATGGTTG
consePGIintUNTR2000	ACATG.TGGT	TAACCTTAACA	GGGCTCCGGC	TGTTGCAAAA CACATGGTTG
Consensus	acatg.tggt	taacttaaca	gggctccggc	tggtgcaaaa cacatggttg
	201		PGI int U --->	250
consePGIintUNTDrakka	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consensWesrPGI	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTR113	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTBrapaA	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
ConsePGIintUNTRRH1	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
PGIBo-EM:AF258277	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
PGIBra-EM:AF258278	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTBolera	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTR2000	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
Consensus	ctgtcagcac	taatcttgc	ggtatg	aatt tgtgattaaa tttgtttgt
	251			300
consePGIintUNTDrakka	TGTGACTCTT	T.CTTTCATTG	TTCGTTTTTCG	TACAATAAAC CGAATGTATA
consensWesrPGI	TGTGACTCTT	T.CTTTCATTG	TTCGTTTTTCG	TACAATAAAC CGAATGTATA
consePGIintUNTR113	TGCGACTCTT	T.CTTTCATTG	TTCGTTTTTCG	TACAATAAAC ..AATGTATA
consePGIintUNTBrapaA	TGCGACTCTT	T.CTTTCATTG	TTCGTTTTTCG	TACAATAAAC ..AATGTATA
ConsePGIintUNTRRH1	TGCGACTCTT	T.CTTTCATTG	TTCGTTTTTCG	TACAATAAAC ..AATGTATA
PGIBo-EM:AF258277	TGTGACTCTT	T.CTTTCATTG	TTCGTTTTTCG	TACAATAAAC CGAATGTATA
PGIBra-EM:AF258278	TGTGACTCTT	TTCTTCATTG	TTCGTTTTTCG	TACAATAAAC CGAATGTATA
consePGIintUNTBolera	TG.GACTCTT	T.CTTTCATTG	TTCGTTTTTCG	TACAATAAAC CGAATGTATA
consePGIintUNTR2000	TGTGACTCTT	T.CTTTCATTG	TTCGTTTTTCG	TACAATAAAC CGAATGTATA
Consensus	tg.gactctt	t.cttctattg	ttcgtttttcg	tacaataaac cgaatgtata

Figure 13 (b)

	301		<---	PGIol antL 341	350
consePGIintUNTDrakka	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consensWesrPGI	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consePGIintUNTR113	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consePGIintUNTBrapaA	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
ConsePGIintUNTRRH1	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
PGIBo-EM:AF258277	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
PGIBra-EM:AF258278	ACCTTTTTTAC AAAC TGAA	AT GTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consePGIintUNTBolera	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consePGIintUNTR2000	ATCTTTT.AC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
Consensus	atcttttttac aaactgaa	tt ttctaccggg tctgatgtac a		atgctAGTC	

Figure 14 (a)

201	PGI int U ---->		250
consePGIintUNTDrakka	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
consensWesrPGI	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTR113	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTBrapaA	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
ConsePGIintUNTRRH1	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
PGIBo-EM:AF258277	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
PGIBra-EM:AF258278	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTBolera	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTR2000	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTTGT
Consensus	ctgtcagcac	taatcttgc ggtatg	aatt tgtgattaaa tttgtttgt
	251		300
consePGIintUNTDrakka	TGTGACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	CGAATGTATA
consensWesrPGI	TGTGACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	CGAATGTATA
consePGIintUNTR113	TGCGACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	..AATGTATA
consePGIintUNTBrapaA	TGCGACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	..AATGTATA
ConsePGIintUNTRRH1	TGCGACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	..AATGTATA
PGIBo-EM:AF258277	TGTGACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	CGAATGTATA
PGIBra-EM:AF258278	TGTGACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	CGAATGTATA
consePGIintUNTBolera	TG.GACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	CGAATGTATA
consePGIintUNTR2000	TGTGACTCTT	T.CTTCATTG TTCGTTTTCG TACAATAAAC	CGAATGTATA
Consensus	tg.gactctt	t.cttcattg ttcgttttcg tacaataaac	cgaaatgtata
	ε		ε3
	301		350
consePGIintUNTDrakka	ATCTTTTAC	AAACTGAA TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consensWesrPGI	ATCTTTTAC	AAACTGAA TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consePGIintUNTR113	ATCTTTTAC	AAACTGAA TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consePGIintUNTBrapaA	ATCTTTTAC	AAACTGAA TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
ConsePGIintUNTRRH1	ATCTTTTAC	AAACTGAA TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
PGIBo-EM:AF258277	ATCTTTTAC	AAACTGAA TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
PGIBra-EM:AF258278	ACCTTTTAC	AAACTGAA AT GTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consePGIintUNTBolera	ATCTTTTAC	AAACTGAA TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consePGIintUNTR2000	ATCTTTT.AC	AAACTGAA TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
Consensus	atctttttac	aaactgaa tt ttctaccggg tctgatgtac a	atgctagtc
	ε		
	351		400
consePGIintUNTDrakka	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTACATGTAT	TCAGACAGTA
consensWesrPGI	TCCATGTTCT	TGGGGATCAT GATTTATTTT CT.CATGTAT	TCAGACAGTA
consePGIintUNTR113	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTACATGTGT	TCAGCCAGTA
consePGIintUNTBrapaA	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTACATGTGT	TCAGCCAGTA
ConsePGIintUNTRRH1	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTACATGTGT	TCAGCCAGTA
PGIBo-EM:AF258277	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTACATGTAT	TCAGACAGTA
PGIBra-EM:AF258278	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTACATGTAT	TCAGACAGTA
consePGIintUNTBolera	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTACATGTAT	TCAGACAGTA
consePGIintUNTR2000	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTACATGTAT	TCAGACAGTA
Consensus	TCCATGTTCT	TGGGGATCAT GATTTATTTT CTaCATGTAT	TCAGaCAGTA
		ε 5	ε 6
	401		450
consePGIintUNTDrakka	CAGAAGAAAG	TGTTCAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
consensWesrPGI	CAGAAGAAAG	TGTTCAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
consePGIintUNTR113	CAGAAGAAAG	TGTTTAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
consePGIintUNTBrapaA	CAGAAGAAAG	TGTTTAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
ConsePGIintUNTRRH1	CAGAAGAAAG	TGTTTAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
PGIBo-EM:AF258277	CAGAAGAAAG	TATTTAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
PGIBra-EM:AF258278	CAGAAGAAA	TGTTTAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
consePGIintUNTBolera	CAGAAGAAAG	TGTTCAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
consePGIintUNTR2000	CAGAAGAAAG	TGTTCAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
Consensus	CAGAAGAAAg	TgTTCAAAC TCTGGATGTT TTAATTTACA	GTTAGTGGAG
	ε 7.		ε

Figure 14 (b)

	451	end of Data Base PGI sequences				500
consePGIintUNTDrakka	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
consensWesrPGI	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
consePGIintUNTR113	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
consePGIintUNTBrapaA	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
ConsePGIintUNTRRH1	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
PGIBo-EM:AF258277	AAGTTCGGCA	TTGATCC...	
PGIBra-EM:AF258278	AAGTTCGGCA	TTGATCCGAA	CAA.....	
consePGIintUNTBolera	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
consePGIintUNTR2000	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
Consensus	AAGTTCGGCA	TTGATCCgaa	caatgcattt	gcattttggg	actggggttg	
	501					550
consePGIintUNTDrakka	TGGAAGGTAC	AGTGGTAAGT	GCTTGTTTAT	TTGGTTGTAT	AAATTTCTCG	
consensWesrPGI	TGGAAGGTAC	AGTGGTAAGT	GCTTGTTTAT	TTGGTTGTAT	AAATTTCTCG	
consePGIintUNTR113	TGGAAGGTAC	AGTGGTAAGT	GCTTGTTTAT	TTGGTTGTAT	TAATTTCTCA	
consePGIintUNTBrapaA	TGGAAGGTAC	AGTGGTAAGT	GCTTGTTTAT	TTGGTTGTAT	TAATTTCTCA	
ConsePGIintUNTRRH1	TGGAAGGTAC	AGTGGTAAGT	GCTTGTTTAT	TTGGTTGTAT	TAATTTCTCA	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	TGGAAGGTAC	AGTGGTAAGT	GCTTGTTTAT	TTGGTTGTAT	AAATTTCTCG	
consePGIintUNTR2000	TGGAAGGTAC	AGTGGTAAGT	GCTTGTTTAT	TTGGTTGTAT	AAATTTCTCG	
Consensus	tggaaggtac	agtggtaagt	gcttgtttat	ttggttgtat	aattttctc	8 9
	551					600
consePGIintUNTDrakka	TCCATTTCCG	CTTGCTTAGT	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
consensWesrPGI	TCCATTTCCG	CTTGCTTAGT	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
consePGIintUNTR113	TCCATATCCG	CTTGCTTAGT	TTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
consePGIintUNTBrapaA	TCCATATCCG	CTTGCTTAGT	TTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
ConsePGIintUNTRRH1	TCCATATCCG	CTTGCTTAGT	TTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	TCCATTTCCG	CTTGCTTAGT	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
consePGIintUNTR2000	TCCATTTCCG	CTTGCTTAGT	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
Consensus	tccatTTccg	cttgcttagt	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	10 11
	601					650
consePGIintUNTDrakka	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCTGTGGTTG	
consensWesrPGI	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCTGTGGTTG	
consePGIintUNTR113	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCCGTGGTTG	
consePGIintUNTBrapaA	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCCGTGGTTG	
ConsePGIintUNTRRH1	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCCGTGGTTG	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCTGTGGTTG	
consePGIintUNTR2000	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCTGTGGTTG	
Consensus	tgctgttgga	gtcttaccat	tgtctctaca	gtatggcttc	tcTgtggttg	12
	651					700
consePGIintUNTDrakka	AGAAGTACGG	TACCTTCTAC	TTTATCAGCC	ATCTCATAAA	ATGTCTTAGG	
consensWesrPGI	AGAAGTACGG	TACCTTCTAC	TTTATCAGCC	ATCTCATAAA	ATGTCTTAGG	
consePGIintUNTR113	AGAAGTACGG	TACCTTCTAC	TTTATTAGCC	ATCTCATAAA	ATGTCTTGGG	
consePGIintUNTBrapaA	AGAAGTACGG	TACCTTCTAC	TTTATTAGCC	ATCTCATAAA	ATGTCTTGGG	
ConsePGIintUNTRRH1	AGAAGTACGG	TACCTTCTAC	TTTATTAGCC	ATCTCATAAA	ATGTCTTGGG	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	AGAAGTACGG	TACCTTCTAC	TTTATCAGCC	ATCTCATAAA	ATGTCTTAGG	
consePGIintUNTR2000	AGAAGTACGG	TACCTTCTAC	TTTATCAGCC	ATCTCATAAA	ATGTCTTAGG	
Consensus	agaagtacgg	taccttctac	tttatCagcc	atctcataaa	atgtcttAgg	13 14

Figure 14 (c)

	701				750
consePGIintUNTDrakka	CATATTCTTT	CTATTTTATT	TTCCTCTTAA	TGATTTCCTC	TTTTTTTTAT
consensWesrPGI	CATATTCTTT	CTATTTTATT	TTCCTCTTAA	TGATTTCCTC	TTTTTTTTAT
consePGIintUNTR113	CATATTCTTT	CTATTTTATT	TTCCTCTGAA	TGATTTCCTC	TCTTTTAT..
consePGIintUNTBrapaA	CATATTCTTT	CTATTTTATT	TTCCTCTGAA	TGATTTCCTC	TCTTTTAT..
ConsePGIintUNTRRH1	CATATTCTTT	CTATTTTATT	TTCCTCTGAA	TGATTTCCTC	TCTTTTAT..
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera	CATATTCTTT	CTATTTTATT	TTCCTCTTAA	TGATTTCCTC	TTTTTTTA..
consePGIintUNTR2000	CATATTCTTT	CTATTTTATT	TCCCTCTTAA	TGATTTCCTC	TTTTTTTTAT
Consensus	catattcctt	ctattttatt	ttcctctaa	tgatttcctc	t.tttt.t..
			15		16 17
	751				800
consePGIintUNTDrakka	TGCATTCCCG	TTTTATTTTC	AAAAGTTGTT	ACTGTCCTA	AATCAAGAAG
consensWesrPGI	TGCATTCCCG	TTTTATTTTC	AAAAGTTGTT	ACTGTCCTA	AATCAAGAAG
consePGIintUNTR113	TGCATTCCCG	TTTTATTTTC	AAAAGTTGTC	ACTGTCCTA	AATCAAGAAG
consePGIintUNTBrapaA	TGCATTCCCG	TTTTATTTTC	AAAAGTTGTC	ACTGTCCTA	AATCAAGAAG
ConsePGIintUNTRRH1	TGCATTCCCG	TTTTATTTTC	AAAAGTTGTC	ACTGTCCTA	AATCAAGAAG
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera	TGCATTCCCG	TTTTATTT.C	AAAAGTTGTC	CGGCCCTA	AACCAAGAAG
consePGIintUNTR2000	TGCATTCCCG	TTTTATTTTC	AAAAGTTGTT	ACTGTCCTA	AATCAAGAAG
Consensus	tgcatcccg	ttttattttc	aaaagttgt.	actgtctcta	aatcaagaag
	801				850
consePGIintUNTDrakka	AAACCTTCTT	AGTAGATCCA	GCTGATATTC	AGCCTTTTCT	AAATTGGACT
consensWesrPGI	AAACCTTCTT	AGTAGATCCA	GCTGATATTC	AGCCTTTTCT	AAATTGGACT
consePGIintUNTR113	AAACCTTCTT	AGTAGATCCA	GTTGATATTC	AGCCTTTTCT	AAATTGGACT
consePGIintUNTBrapaA	AAACCTTCTT	AGTAGATCCA	G.TGATATTC	AGCCTTTTCT	AAATTGGACT
ConsePGIintUNTRRH1	AAACCTTCTT	AGTAGATCCA	GTTGATATTC	AGCCTTTTCT	AAATTGGACT
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera	AAACCTTCTT	AGGA...CCA	GA...CTCC	ACCCTTTTCT	AAATTGGACT
consePGIintUNTR2000	AAACCTTCTT	AGTAGATCCA	GCTGATATTC	AGCCTTTTCT	AAATTGGACT
Consensus	aaaccttctt	agtagatcca	g.tgatattc	agccttttct	aaattggact
			18		19
	851				900
consePGIintUNTDrakka	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCCCC
consensWesrPGI	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCCAC
consePGIintUNTR113	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCC..
consePGIintUNTBrapaA	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCC..
ConsePGIintUNTRRH1	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCCAC
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera	GCAGGTTTTT	AAA.GGGGCG	TTCAAGCATT	GATCAGCATT	TCCAGTCCAC
consePGIintUNTR2000	GCAGGTTTTT	AAACGGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCCAC
Consensus	gcagggtttt	aaa.gggagc	ttcaagcatt	gatcagcatt	tccagtcc.c
				20	
	901				950
consePGIintUNTDrakka	ACC.GTTTGA	GAAGAATATA	CCCGTGAGTT	GCATTAGTTT	GTGTGATTAT
consensWesrPGI	ACC.GTTTGA	GAAGAATATA	CCCGTGAGTT	GCATTAGTTT	GTGTGATTAT
consePGIintUNTR113	.CCCGTTTGA	GAAGAATATA	CCCGTGAGTT	GCATTAGTTT	..GTGATTAT
consePGIintUNTBrapaA	.CCCGTTTGA	GAAGAATATA	CCCGTGAGTT	GCATTAGTTT	GTGTGATTAT
ConsePGIintUNTRRH1	ACC.GTTTGA	GAAGAATATA	CCCGTGAGTT	GCATTAGTTT	GTGTGATTAT
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera	ACCCGTTTGA	GAAGAATATA	CCCGTGAGTT	GCATTAGTTT	GTGTGATTAT
consePGIintUNTR2000	ACC.GTTTGA	GAAGAATATA	CCCGTGAGTT	GCATTAGTTT	GTGTGATTAT
Consensus	acc.gtttga	gaagaatata	cccgtagatt	gcattagttt	gtgtgattat

Figure 14 (d)

	951				1000
consePGIintUNTDrakka	ACAGTTTTTC	TTGTCTTTTT	GCTATGCCCA	TCAACACTAG	AAGATTCCTG
consensWesrPGI	ACAGTTTT.C	TTGTCTTTT.	GCTATGTCCA	TCAACACTAG	A.GATTCCTG
consePGIintUNTR113	ACAGTTTT.C	TTGCCTTTTT	GCTAT..AGG	GCAAC.CTAG	A.GATTCATG
consePGIintUNTBrapaA	ACAGTTTT.C	TTGTCTTTTT	GCTATG.TCA	TCAAC.CTAG	A.GATTCATG
ConsePGIintUNTRRH1	ACAGTTTT.C	TTGTCTTTTT	GCTAT...AT	GCAACCCTAG	..GATTCATG
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera	ACAGTTTT.C	TTGTCTTTTT	GCTAG..TGA	TCAAC.CTAG	A.GATTCCTG
consePGIintUNTR2000	ACAGTTTT.C	TTGTCTTTTT	GCTATGTCCA	TCAACACTAG	A.GATTCCTG
Consensus	acagtttt.c	ttgtcttttt	gctat....a	tcaac.ctag	a.gattcctg
					21

	1001				1050
consePGIintUNTDrakka	AAGTTATTAG	TGTAGCCAAC	GCCTAGGGGG	AGGTTGGTTG	GCTGTTTTGG
consensWesrPGI	AAGTTATTAG	TGTAGTCAAC	GCA.....
consePGIintUNTR113	AAGTTATTAG	TGTAGTCAAC	GCAGAGGAGA	G..TTCCTG	ACGG.....
consePGIintUNTBrapaA	AAGTTATTAG	TGTAGTCAAC	GCAGAGTGAG	AGG.TGATTG
ConsePGIintUNTRRH1	AAGTTATTAG	TGTAGTCAAC	GCAGAGGAGG	AGATGGTT..
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera	AAGTTATTAG	TGTAGTCAAC	GCATAGGAGG	AGC.....
consePGIintUNTR2000	AAGTTATTAG	TGTAGTCAAC	GCATAGGGAG	AGGTGAT.GG	TGACTTTTGG
Consensus	aagttattag	tgtagtcaac	gca.agg.g.	.g.....

	1051		1076	
consePGIintUNTDrakka	ACGTTTTTAC	GTGCTCCGGG	GGGTTTTTGG	GGACCAAACC
consensWesrPGI
consePGIintUNTR113
consePGIintUNTBrapaA
ConsePGIintUNTRRH1
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera
consePGIintUNTR2000	ACGATTTCAG	GTGCTTTAGG	GTTATTG

Figure 15 (a)

	51	100
EMBH44836anti
GCP18-5CP418L-Sams
GCP18-2CP418L-Wes
GCP18-4CP418L-R2000	CP418L]...
conse129ba1-Drak	AAACAAATCA AAATTCTAA TTTCTCCA
GCP18-129Sam-ba2	AAAC TATGTA ACAAAATCA AAATTGTAAA TGTCTCCA
GCP18-3129R211-ba2	AA CCAAAATCC AAATTGTAAA TGTCTCCT.
GCP18-10129R20-ba2	CAAAATCCA AAATTGTAAA TGTCTCCT
Consensus
	101	150
EMBH44836anti
GCP18-5CP418L-SamsAT A.CATTTTCT GTAA
GCP18-2CP418L-WesAGG T.AT A.CATTTTCT GTAA
GCP18-4CP418L-R2000AGG TCAT A.CATTTTCT GTAA
conse129ba1-Drak	TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTT CT GTAA	
GCP18-129Sam-ba2	TCCTAACCGAC CTACAGAATA GAGTTATCAT AACATTTTCT G AA	
GCP18-3129R211-ba2	TGCTAACCGC CTCAAAAA.A GAGGTATCAA AAC.TTTTCT GT.A	
GCP18-10129R20-ba2	TGGTTACCGC C.CAAAAA.A AAGGT..CAA AACTT.TCCG GTAA	
Consensus
	151	200
EMBH44836anti
GCP18-5CP418L-Sams	.TATTTCCAT CAAAATGA..	.CTAGAGAAC AGCAGTTCTT ATAACATTAT
GCP18-2CP418L-Wes	.TATTTCCAT CAAAATGA..	.CTAGAGAAC AG.AGTTCCTT ATAACATTAT
GCP18-4CP418L-R2000	ATATTTCCAT CAAAATGA..	.CTAGAGAAC AG.AGTTCCTT ATAACATTAT
conse129ba1-Drak	ATATTTCCAT CAAAATGA..	.CTAGAGAAC AG.AGTTCCTT ATAACATTAT
GCP18-129Sam-ba2	ATGTTTCCAT CAAAATGA	CTATCGAAC ATAATTAAT ATA.CATTT
GCP18-3129R211-ba2	ATGTTTCCAT CAAAATG.	CTATCGGAC ATAATTAAT ATAAC.TTCT
GCP18-10129R20-ba2	ATGTTTCCAT CAAAATG.	CTTCGGA.C ATAATTAAT ATAAC.TTCT
Consensus	ATGTTTCCCT CAAA.TGG	CTTCGGA.C ATAATTAAA A...CATTC
	201	250
EMBH44836anti
GCP18-5CP418L-Sams	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT	
GCP18-2CP418L-Wes	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC ATTATAACAT	
GCP18-4CP418L-R2000	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT	
conse129ba1-Drak	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT	
GCP18-129Sam-ba2	CTG. AAAAT.ATTCCCT CAAAA TTA. .CATTT TTC TTACAA.A.	
GCP18-3129R211-ba2	CTG. AAAAT.ATTCCCT CAAAA TTA. ACATTT TTC T.ACAA.A.	
GCP18-10129R20-ba2	CTG. AAA.TAATTCCCT CAAAA TTA. ACAT. TTC T.ACAA.A.	
Consensus
	251	300
EMBH44836antiCTATACC
GCP18-5CP418L-Sams	TGCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAG CTCCTATAAC	
GCP18-2CP418L-Wes	TGCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAG CTCCTATAAC	
GCP18-4CP418L-R2000	TGCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAG CTCCTATAAC	
conse129ba1-Drak	TGCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAG CTCCTATAAC	
GCP18-129Sam-ba2	TGTTTC... .. CATCAAAATG AGACTCA.G	
GCP18-3129R211-ba2	TGTTTC... .. CATCAAAATG AGACTCA.G	
GCP18-10129R20-ba2	TGTTTC... .. CATCAAAATG AGACTCA.G	
Consensus	tttctgt.aa tgtttccatc aaaatgacta tcgaacataa ttaatAtaac	
	301	350
EMBH44836anti	A TTGTTT ATACAAAGTT TCACT AAAT CTACAAACTT CCCCCGTAAA	
GCP18-5CP418L-Sams	A TTGTTT ATACAAAGTTT .CACT AAAT CTACAAACTT TCCCCGTAAA	
GCP18-2CP418L-Wes	A TTGTTT ATACAAAGTT TCACT AAAT CTACAAACTT TCCCCGTAAA	
GCP18-4CP418L-R2000	A TTGTTT ATACAAAGTTT .CACT AAAT CTACAAACTT TCCCCGTAAA	
conse129ba1-Drak	A. TGTTT ATACAAAGTT TCACT AAAT CTACAAACTT TCCCCGTAAA	
GCP18-129Sam-ba2	ATC.CAGTTC TTGCATAGTT TCACTAAAT CTACAAACTT TC.....	
GCP18-3129R211-ba2	ATCAGTTC TTGCATAGTT TCACT.AAAT CTACAAACTT TC.....	
GCP18-10129R20-ba2	A.CCCAGTTC TTGCATAGTT TC.CT.AAAT CTACAAACTT TC.....	
Consensus

Figure 15 (b)

351 400

EMBH44836anti TGAGCTTAAT ATCACCCTAA. GATGTTTCA ATCAGAT AAA GAGTAACGAC
 GCP18-5CP418L-Sams TGAGCTTAAT ATCACCCTAA GATGTTTCA ATCAGAT AAA GAGTAACGAC
 GCP18-2CP418L-Wes TGAGCTTAAT ATCACCCTAA GATGTTTCA ATCAGAT AAA GAGTAACGAC
 GCP18-4CP418L-R2000 TGAGCTTAAT ATCACCCTAA GATGTTTCA ATCAGAT AAA GAGTAACGAC
 conse129ba1-Drak TGAGCTTAAT ATCACCCTAA GATGTTTCA ATCAGAT AAA GAGTA.CGAC
 GCP18-129Sam-ba2AAT CTTATCTTAA G.TTATCAC ATCAGAT GAA GA.....
 GCP18-3129R211-ba2AAT CTTATCTTAA. G.TTATCAC ATCAGAT GAA GA.....
 GCP18-10129R20-ba2AAT CTTATCTTAA G.TTATCAC ATCAGAT GAA GA.....
 Consensus

401

EMBH44836anti ATCGTTTTGA GATTAGAACA AA
 GCP18-5CP418L-Sams ATCGTTTTGA GATTAGAACA AA
 GCP18-2CP418L-Wes ATCGTTTTGA GATTAGAACA AA
 GCP18-4CP418L-R2000 ATCGTTTTGA GATTAGAACA AA
 conse129ba1-Drak ATCGTTTTGA GATTAGAACA AA
 GCP18-129Sam-ba2GAGC AA
 GCP18-3129R211-ba2GAGC AA
 GCP18-10129R20-ba2GAGC A.
 Consensus

431 480

EMBH44836anti CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
 GCP18-5CP418L-Sams CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
 GCP18-2CP418L-Wes CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAG.
 GCP18-4CP418L-R2000 CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
 conse129ba1-Drak CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
 GCP18-129Sam-ba2 GTAAACCTTA CTTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
 GCP18-3129R211-ba2 GTAAACCTTA CTTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
 GCP18-10129R20-ba2 GTAA.CCTTA CTTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
 Consensus .t.aa.ctta c.tagagtga t.tgaggagt aggctcgttg ccagc.gagc

481 530

EMBH44836anti TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGACCTG AGACAACCGT
 GCP18-5CP418L-Sams TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGACCTG AGACAACCGT
 GCP18-2CP418L-Wes TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGACCTG AGACAACCGT
 GCP18-4CP418L-R2000 TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGACCTG AGACAACCGT
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 GCP18-3129R211-ba2 TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGACCTG AGACAACCGT
 GCP18-10129R20-ba2 TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGACCTG AGACA.CCGT
 Consensus tagctctctc ctccgcctca tgaagcatct g.tgcacctg agacaacctg

531 580

EMBH44836anti GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
 GCP18-5CP418L-Sams GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
 GCP18-2CP418L-Wes GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
 GCP18-4CP418L-R2000 GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
 conse129ba1-Drak GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
 GCP18-129Sam-ba2 GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
 GCP18-3129R211-ba2 GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
 GCP18-10129R20-ba2 GACGAAACTT TCCGATCCCC GCC.CCAGAA TTCGACGCCG CGCATCGGAA
 Consensus gacgaaactt tccgatcacc gccaccagaa ttcgacgccg cgcacgga

581 630

EMBH44836anti GGATCCGAAT CGGGAACCTG AGTGAACCCG AGCGATCCCG GGAGTGCAGC
 GCP18-5CP418L-Sams GGATCCGAAT CGGGAACCTG AGTGAACCCG AGCGATCCCG GGAGTGCAGC
 GCP18-2CP418L-Wes GGATCCGAAT CGGGAACCTG AGTGAACCCG AGCGATCCCG GGAGTGCAGC
 GCP18-4CP418L-R2000 GGATCCGAAT CGGGAACCTG AGTGAACCCG AGCGATCCCG GGAGTGCAGC
 conse129ba1-Drak GGATCCGAAT CGGGAACCTG AGTGAACCCG AGCGATCCCG GGAGTGCAGC
 GCP18-129Sam-ba2 GGATCCGAAT CGGGAACCTG AGTGAACCCG AGCGATCCCG GGAGTGCAGC
 GCP18-3129R211-ba2 GGATCCGAAT CGG.AACTGG AGTGAACCCG AGCGATCCCG GGAGTGCAGC
 GCP18-10129R20-ba2 GGATCCGAAT CGGGAACCTG AGTGAACCCG AGCGATCCCG GGAGTGCAGC
 Consensus ggatccgaat cgggaactgg agtgaaccg agcgatcccg ggagtgcgac

Figure 15 (c)

	631		690
EMBH44836anti	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT TCGACGAAGA GTGGAAGAGG
GCP18-5CP418L-Sams	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT TCGACGAAGA GTGGAAGAGG
GCP18-2CP418L-Wes	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT TCGACGAAGA GTGGAAGAGG
GCP18-4CP418L-R2000	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT TCGACGAAGA GTGGAAGAGG
conse129ba1-Drak	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT TCGACGAA.A GTGGAAGAGG
GCPS18-129Sam-ba2	GGAGCGTTGG	AAAAAGAGAG	TGGCACGATT TCGACGAAGA GAGGAAGAGG
GCPR18-3129R211-ba2	GGAGCGTTGG	AAAAAGAGAG	TGGCACGATT TCGACGAAGA GAGGAAGAGG
GCP18-10129R20-ba2	GGAGCGTTGG	AAAAAGAGAG	TGGCACGATT TCG.CGAAGA GAGGAAGAGG
Consensus	ggagcg.tgg	.aaaagagag	tggcacgatt tcgacgaaga g.ggaagagg

	691		740
EMBH44836anti	AGAGGGTGGT	GGATAAACTC	GCGTATGATC AAGTTCGTCA TCGTCCTGAT
GCP18-5CP418L-Sams	AGAGGGTGGT	GGATAAACTC	GCGTATGATC AAGTTCGTCA TCGTCCTGAT
GCP18-2CP418L-Wes	AGAGGGTGGT	GGATAAACTC	GCGTATGATC AAGTTCGTCA TCGTCCTGAT
GCP18-4CP418L-R2000	AGAGGGTGGT	GGATAAACTC	GCGTATGATC AAGTTCGTCA TCGTCCTGAT
conse129ba1-Drak	AGAGGGTGGT	GGATAAACTC	GCGTATGATC AAGTTCGTCA TCGTCCTGAT
GCPS18-129Sam-ba2	AGAGGGTGGT	GGATAAACTC	GCGTATGATC AAGTTCGTCA TCGTCCTGAT
GCPR18-3129R211-ba2	AGAGG.TGGT	GGATAAACTC	GCGTATGATC AAGTTCGTCA TCGTCCTGAA
GCP18-10129R20-ba2	AGAGGGTGGT	GGATAAACTC	GCGTATGATC AAGTTCGTCA TCGTCCTGAA
Consensus	agagggtggt	ggataaaactc	gcgtatgatac aagttcgtca tcgtcctga.

	741		790
EMBH44836anti	TGCCGCCATT	TTTTTTGTCA	GGGCGCTCTG TGGCTTAGAA GTTTCCGATG
GCP18-5CP418L-Sams	TGCCGCCATT	TTTTTTGTCA	GGGCGCTCTG TGGCTTAGAA GTTTCCGATG
GCP18-2CP418L-Wes	TGCCGCCATT	TTTTTTGTCA	GGGCGCTCTG TGGCTTAGAA GTTTCCGATG
GCP18-4CP418L-R2000	TGCCGCCATT	TTTTTTGTCA	GGGCGCTCTG TGGCTTAGAA GTTTCCGATG
conse129ba1-Drak	TGCCGCCATT	TTTTTTGTCA	GGGCGCTCTG .GGCTTAGAA GTTTCCGA..
GCPS18-129Sam-ba2	TGCCGCCATT	CTTGTTTAC.	.GGCGCTCTG GGT.....
GCPR18-3129R211-ba2	TGCCGCC..
GCP18-10129R20-ba2	TGCC..CAT.	CTTGAGCTC.	.GG.GCGCGG GCTCACAA..
Consensus	tgccgccat.	.tt.....c.	.gg.gc.c.g

	791	
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GCP18-5CP418L-Sams	TCAATGAAC	AGAAT...TC CGGG...
GCP18-2CP418L-Wes	CCAATGAACA	AGATTATTC CGATG..
GCP18-4CP418L-R2000
conse129ba1-Drak

800

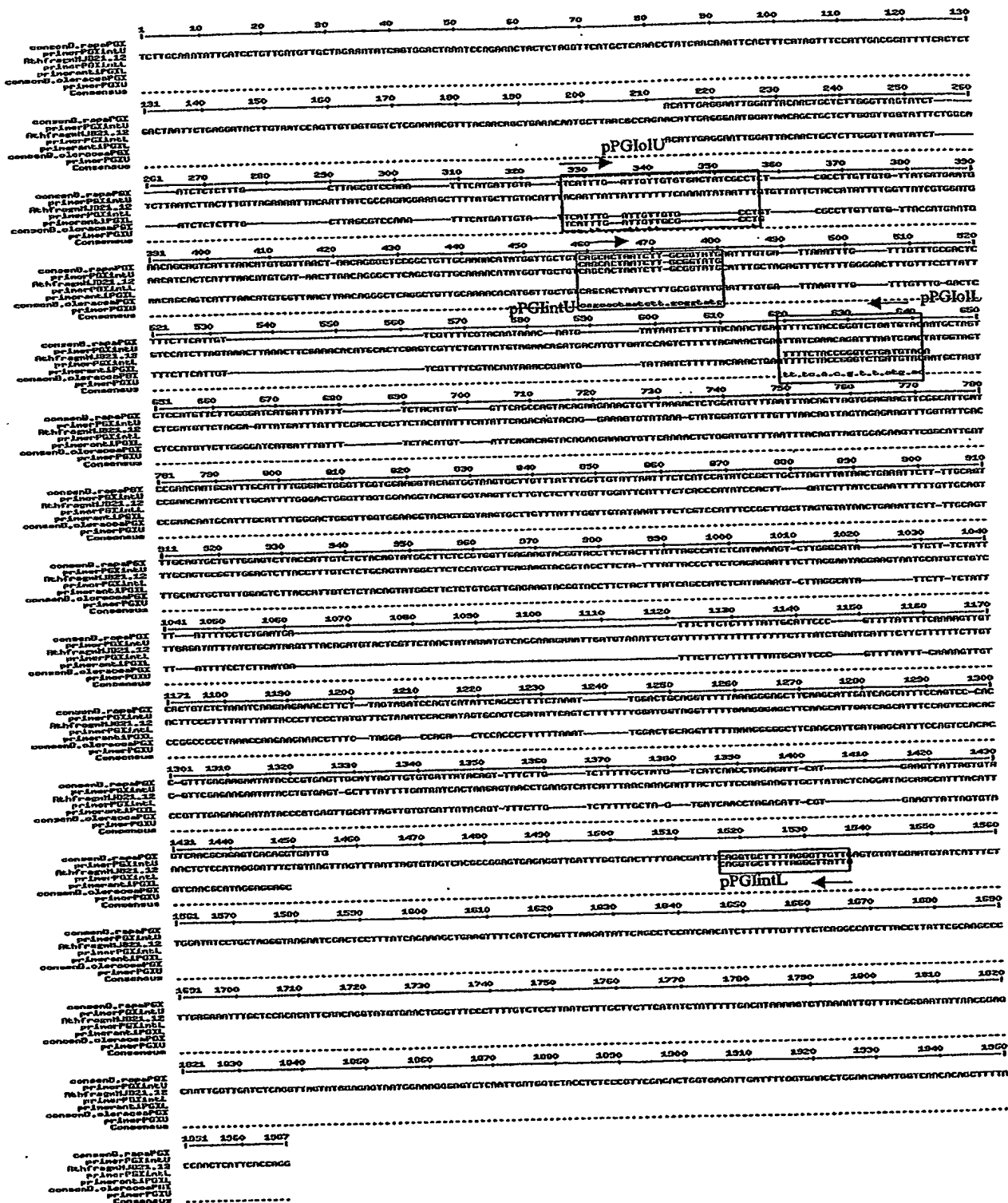
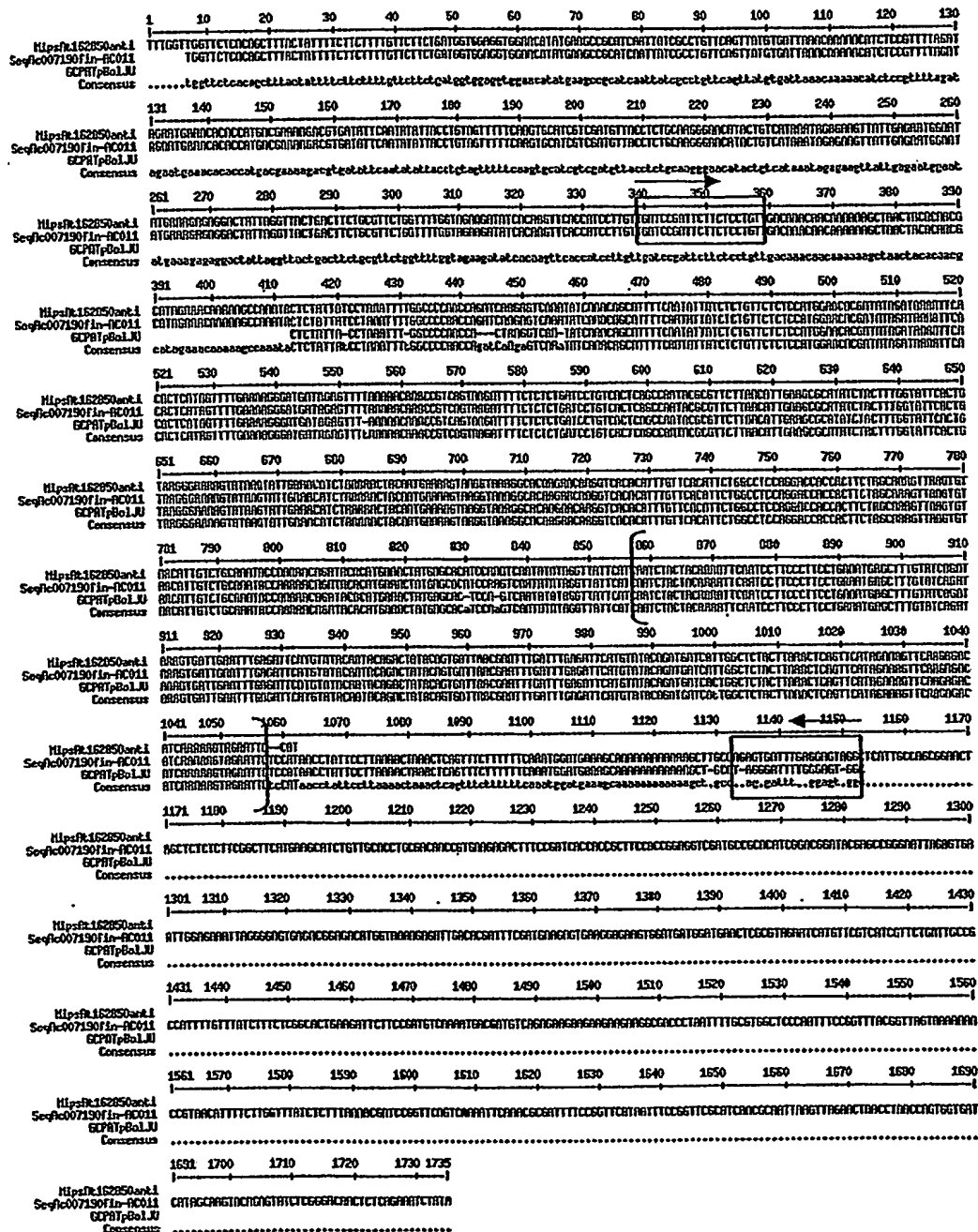


Figure 17



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 having a good agronomic value

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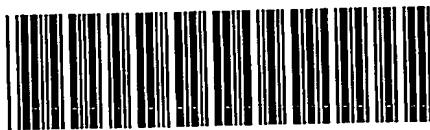
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